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(54) **3D CELL CULTURE VESSELS FOR MANUAL OR AUTOMATIC MEDIA EXCHANGE**

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CPC C12M 23/16; C12M 23/08; C12M 23/12; C12M 23/44
See application file for complete search history.

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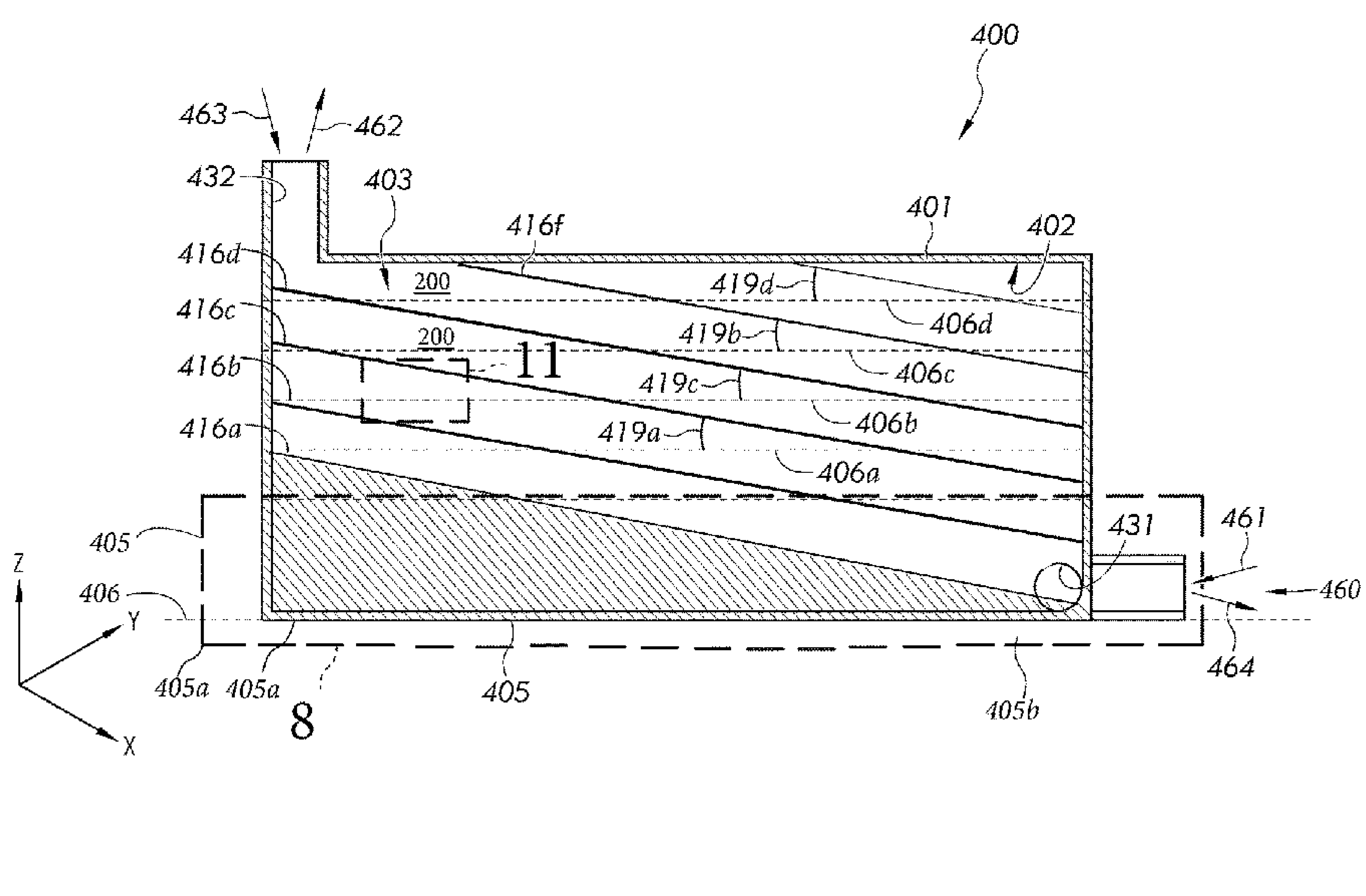
(57) **ABSTRACT**

A cell culture vessel includes a base defining a base plane extending in a first direction and a second direction perpendicular to the first direction, a plurality of cell culture chambers stacked one atop another, each cell culture chamber having a top, a bottom and sidewalls, each of the top, bottom and sidewalls having an interior surface, wherein at least the bottom surface has an array of microcavities supporting the culture of cells as spheroids and each bottom surface is at an angle with respect to the plane of a table or surface upon which the vessel sits. Further, liquid can flow into each cell culture chamber via an inlet and out of each cell culture chamber via an outlet. The angled cell culture surface allows the cell culture chambers to be perfused or allows media changes without dislodging spheroids from microcavities.

27 Claims, 12 Drawing Sheets



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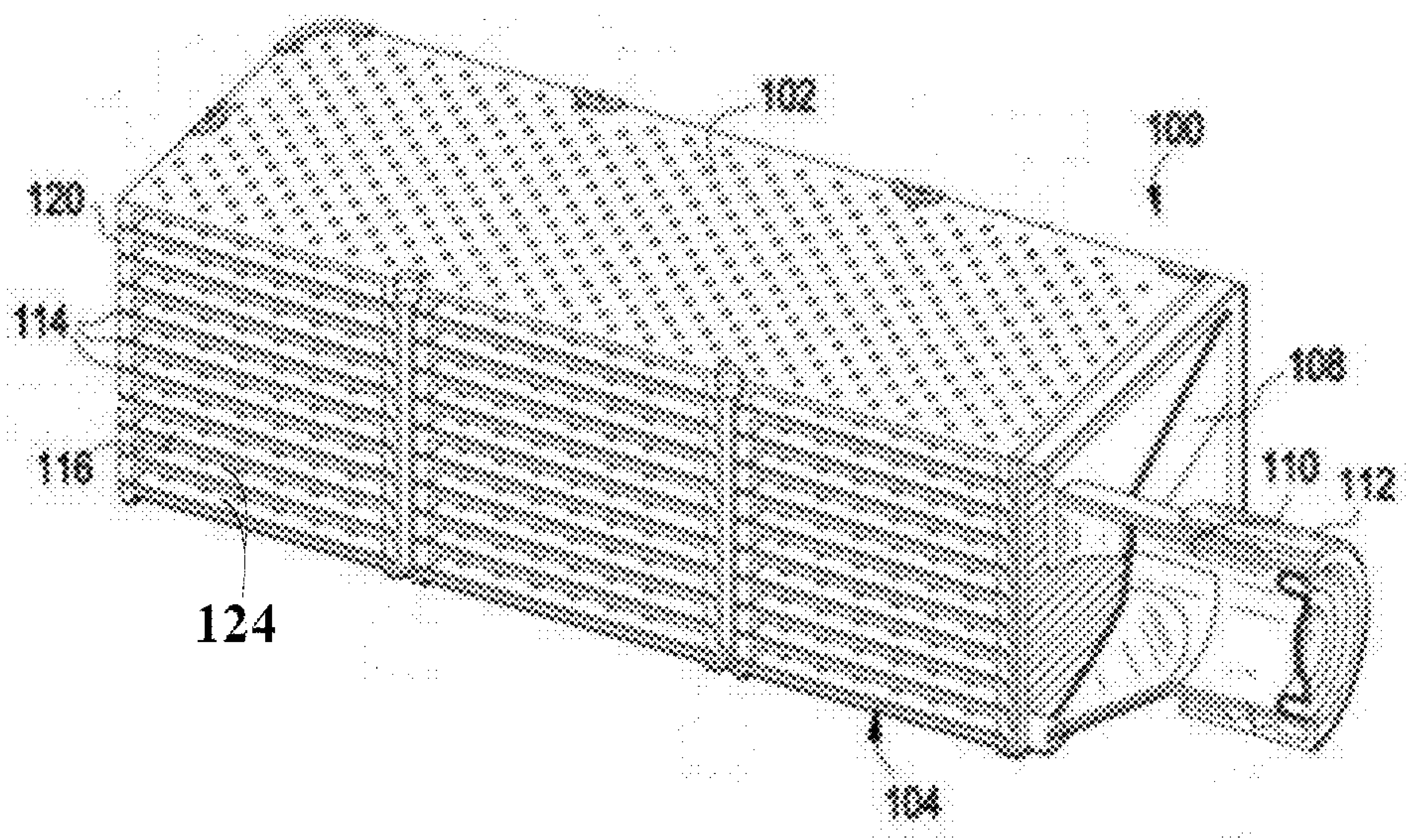

FIG. 1

PRIOR ART

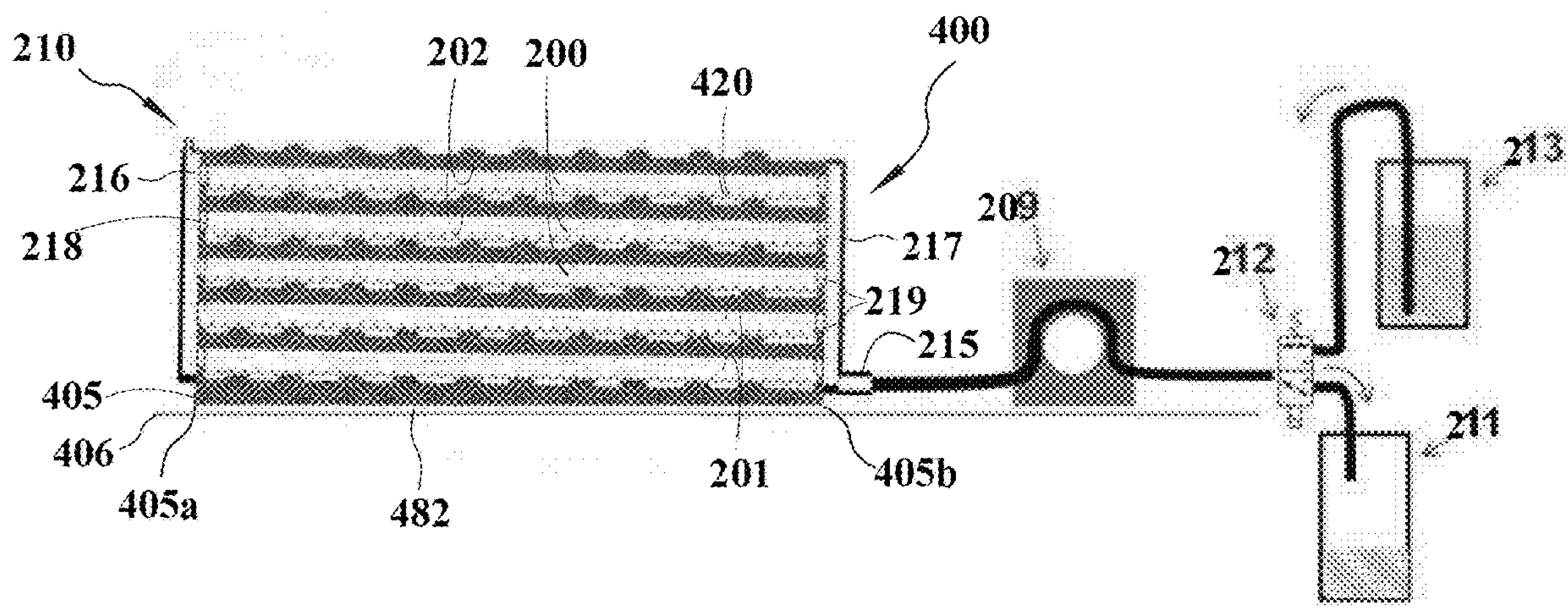
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FIG. 2

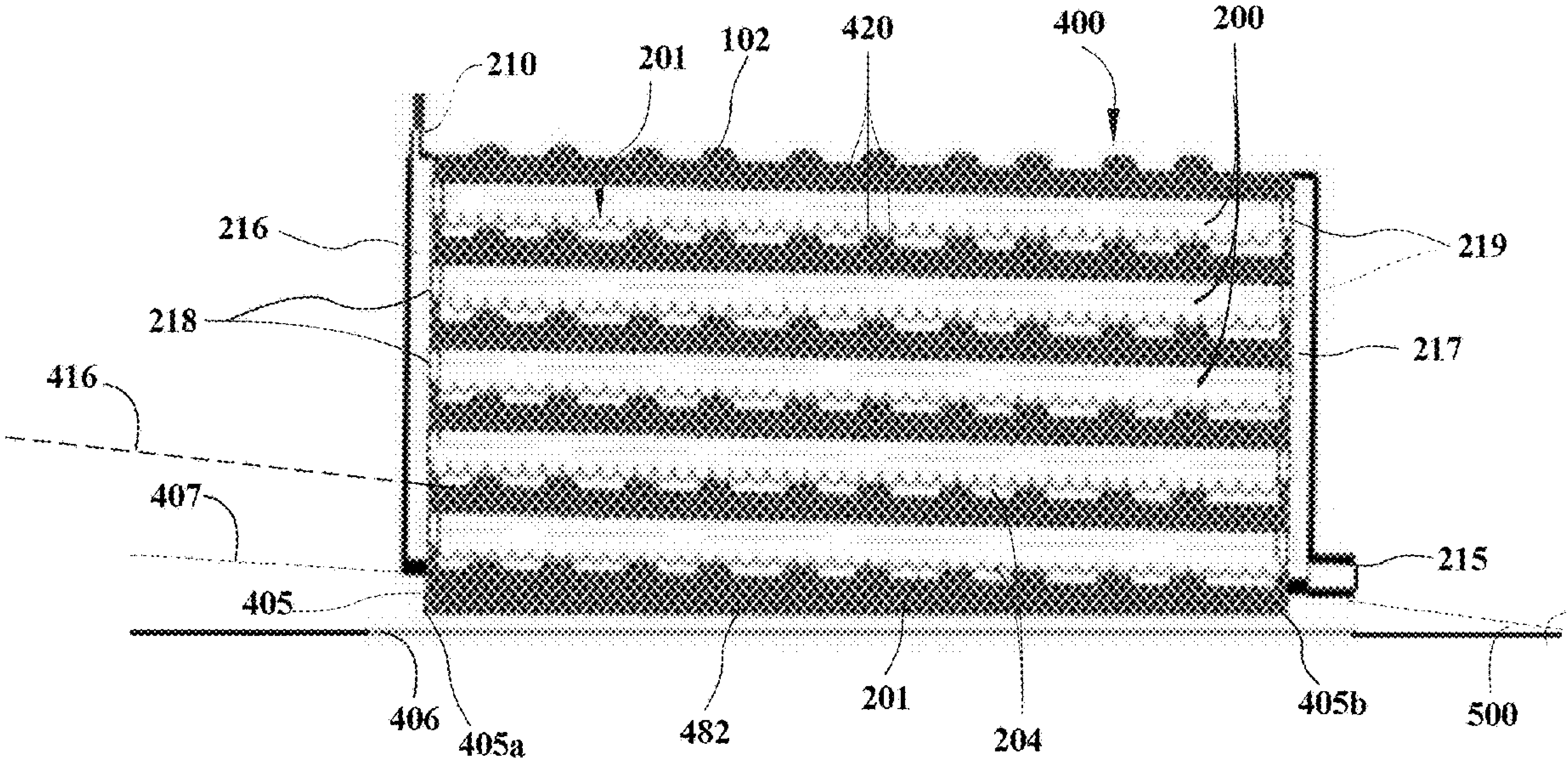
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FIG. 3

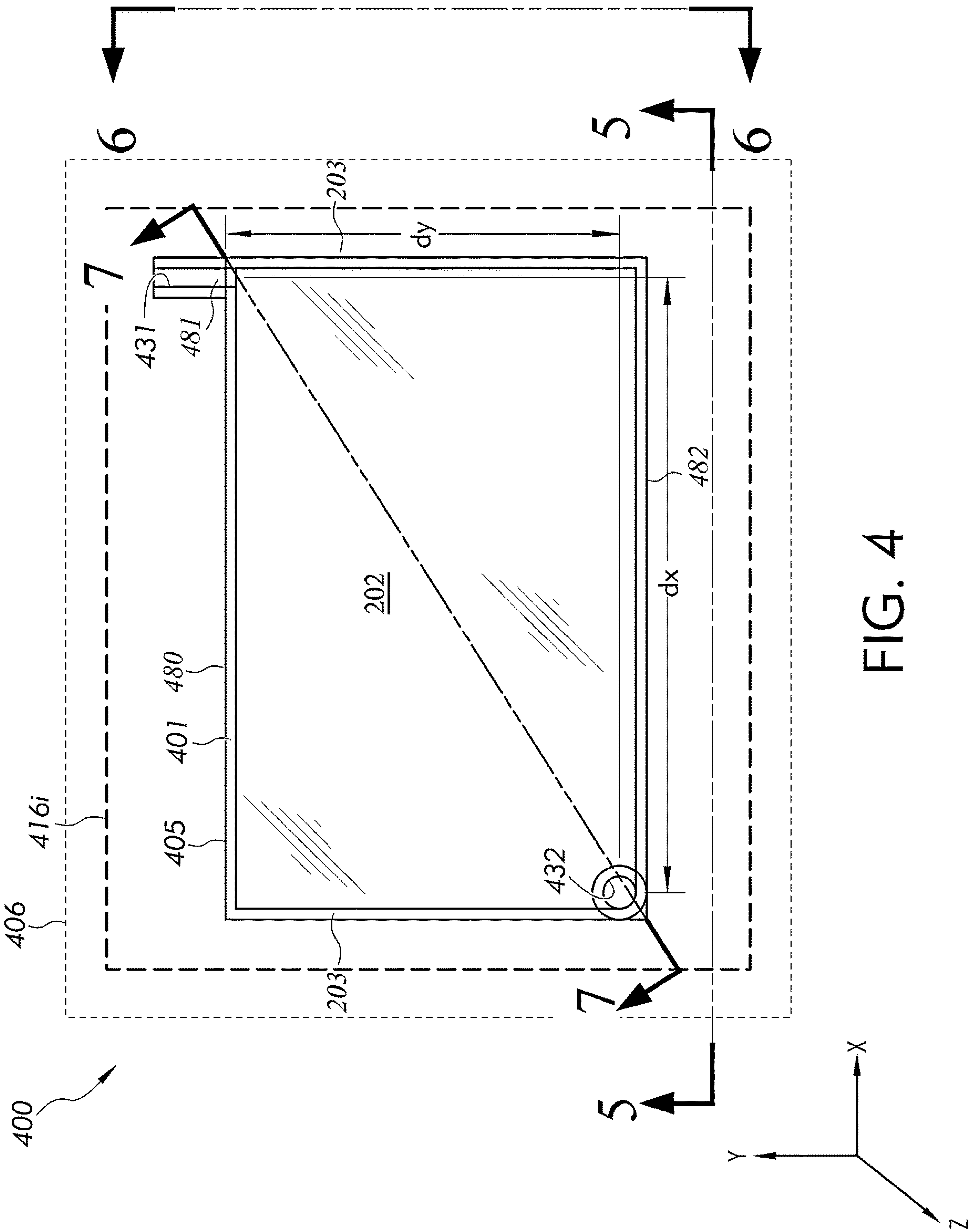

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FIG. 4

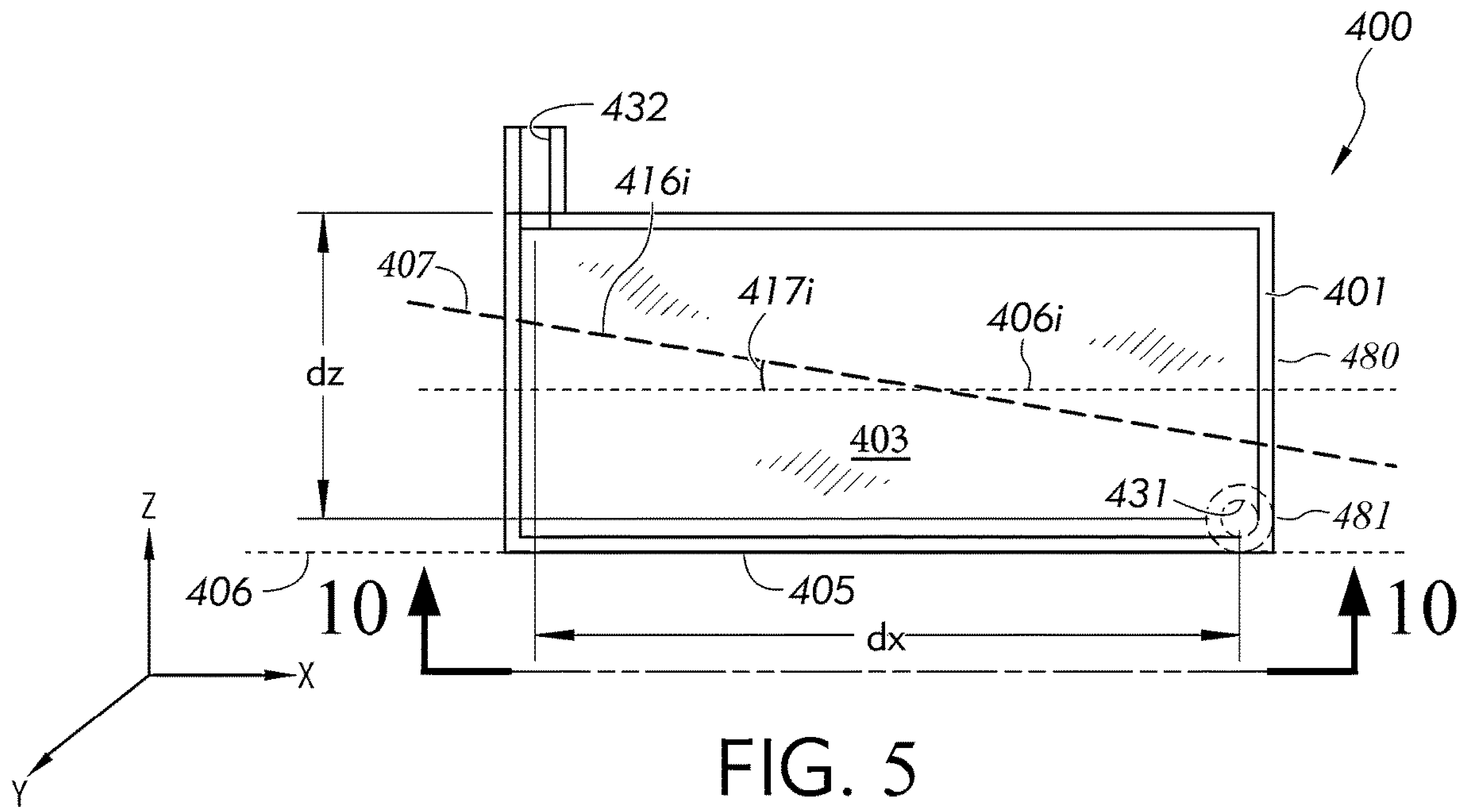

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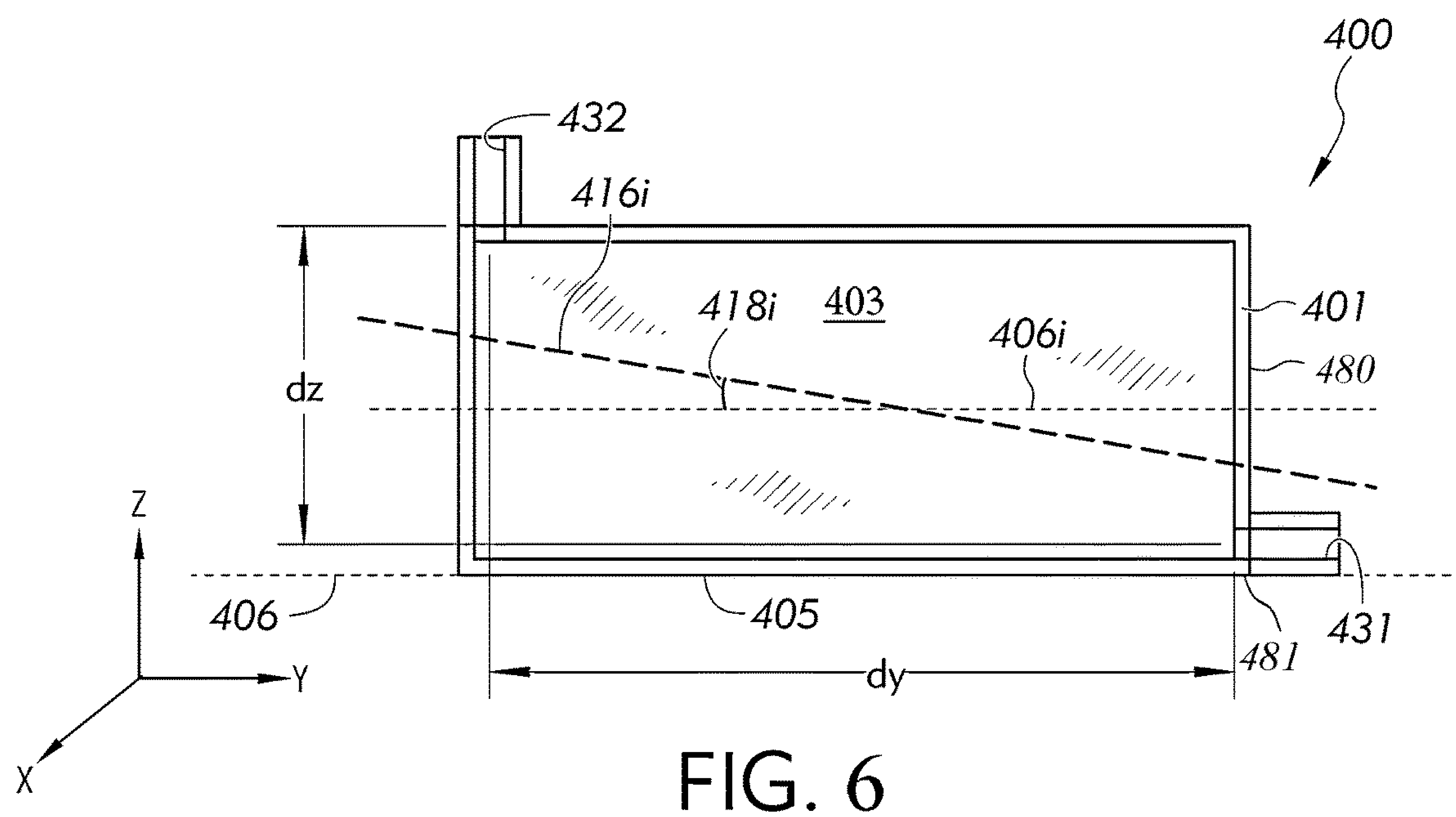
FIG. 5



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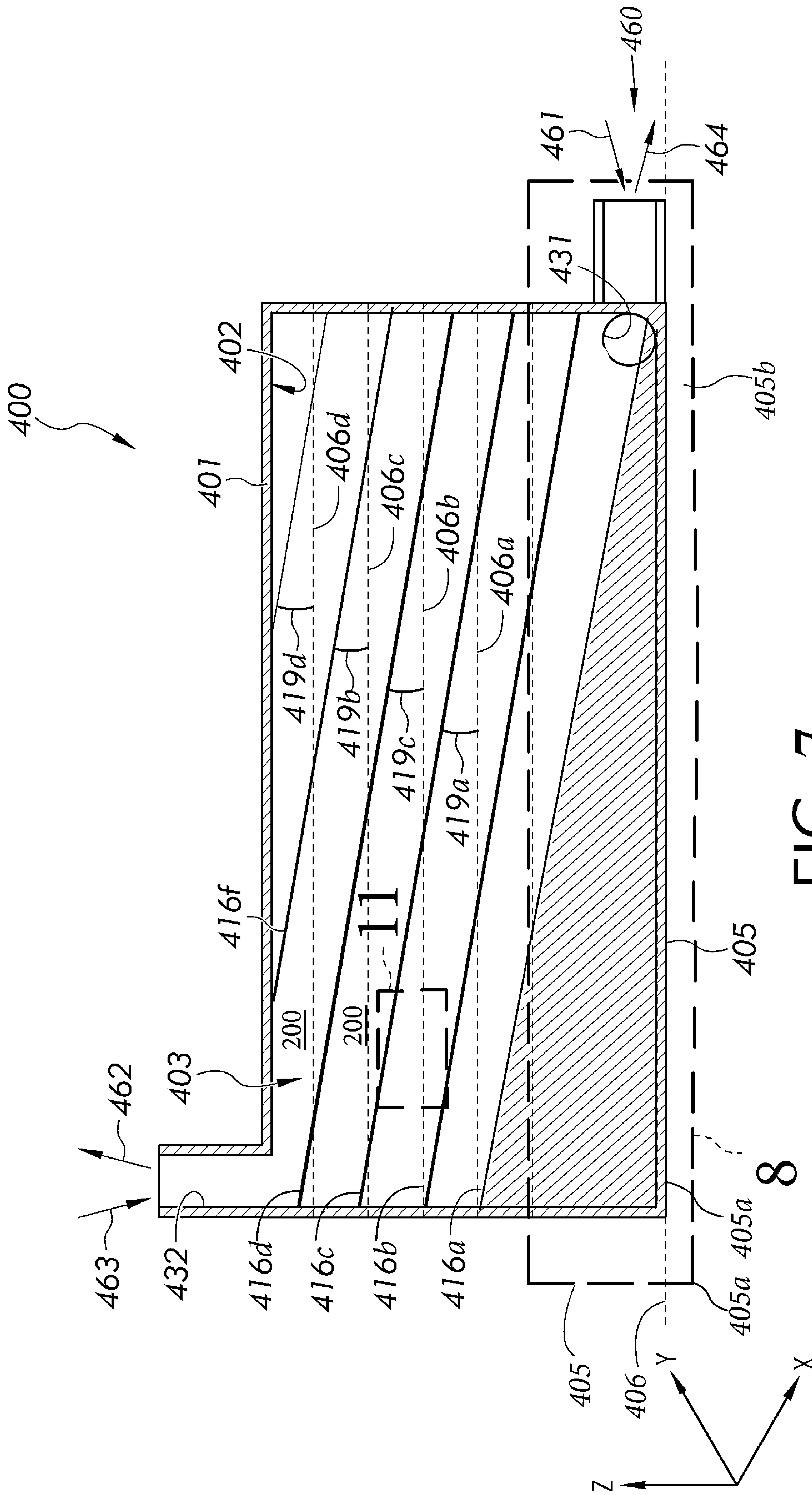
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FIG. 7

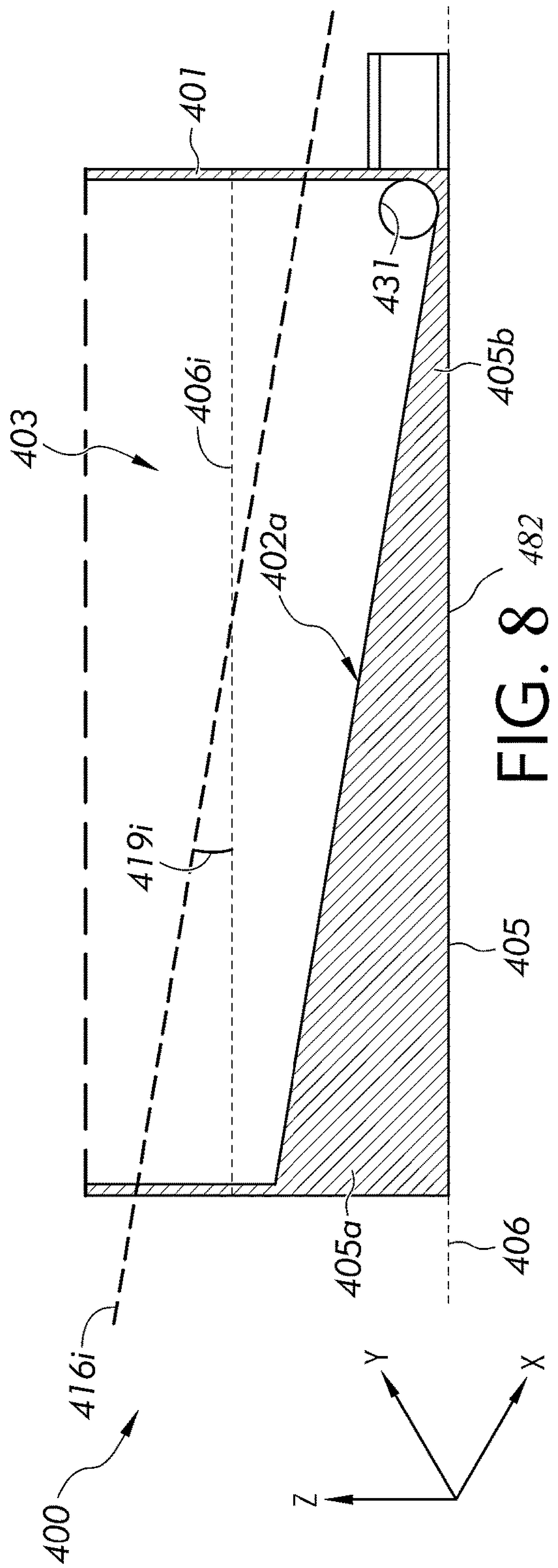
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FIG. 8

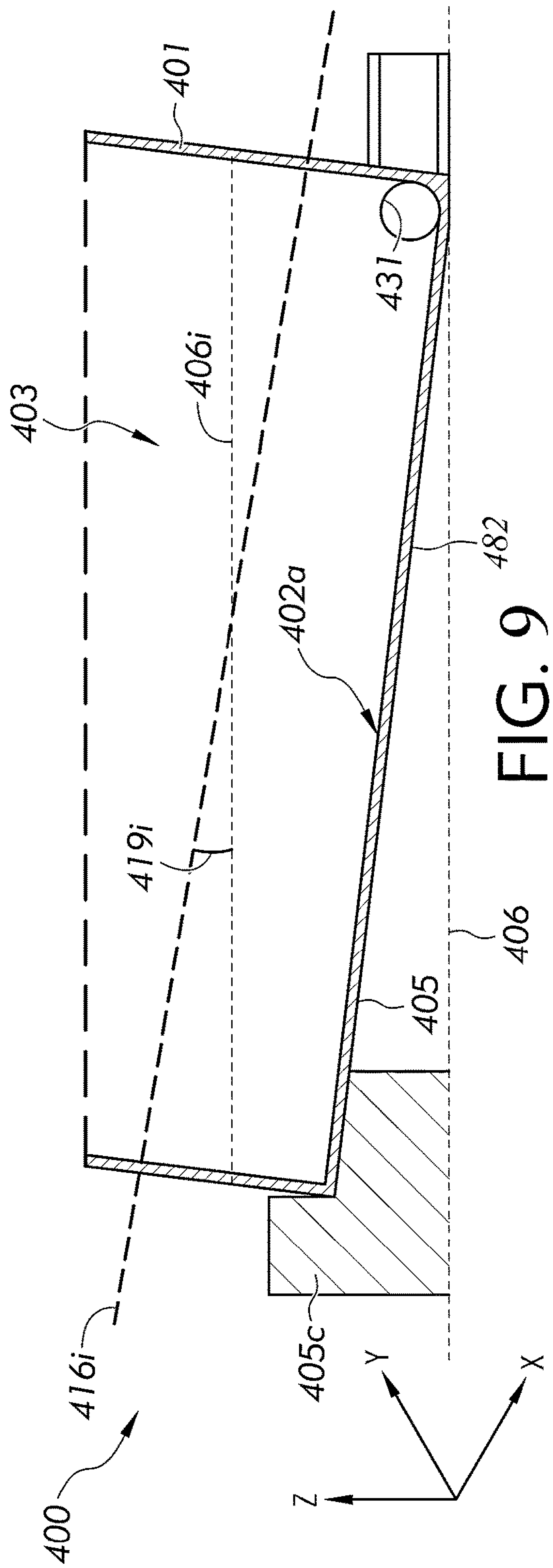
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FIG. 9

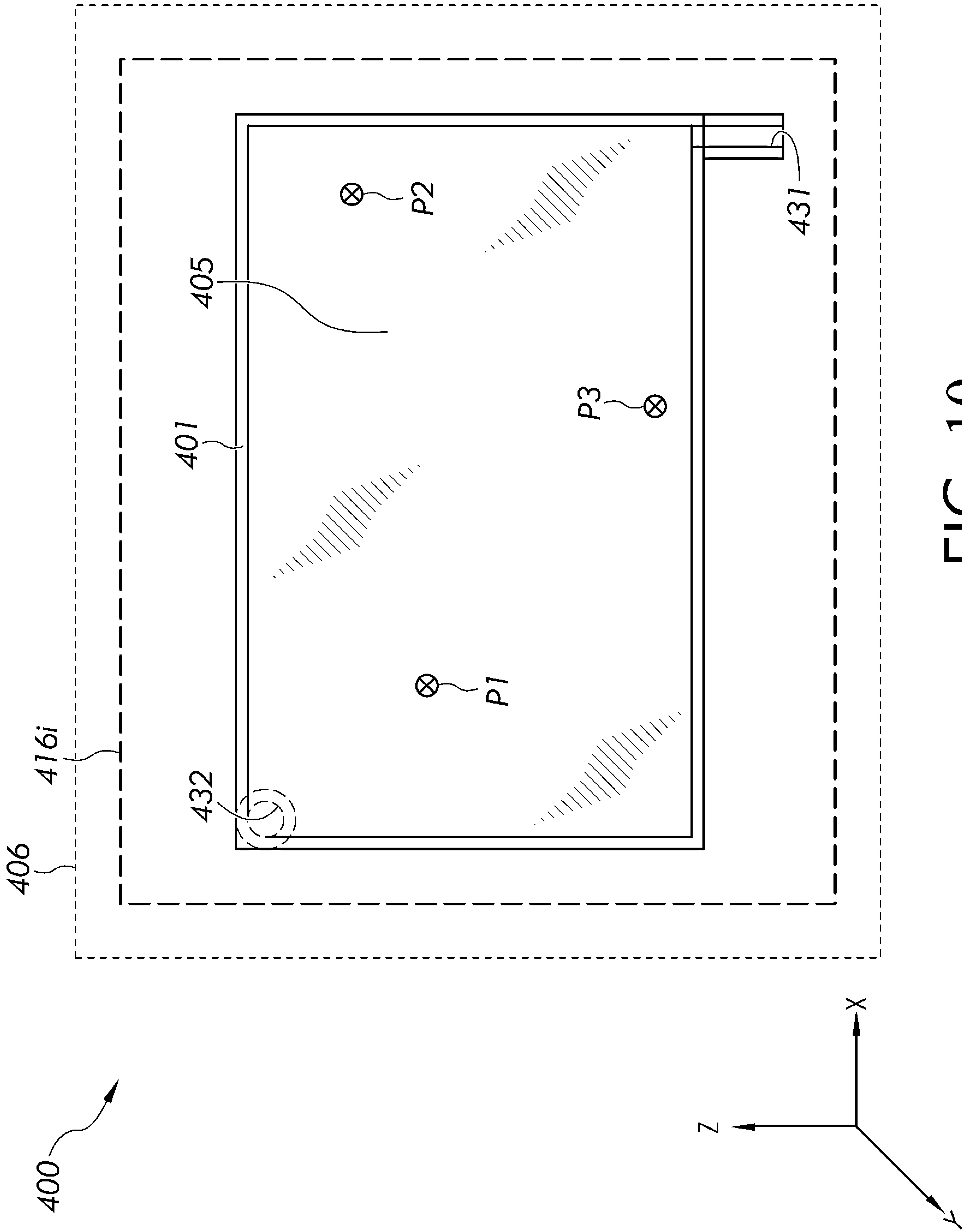

FIG. 10

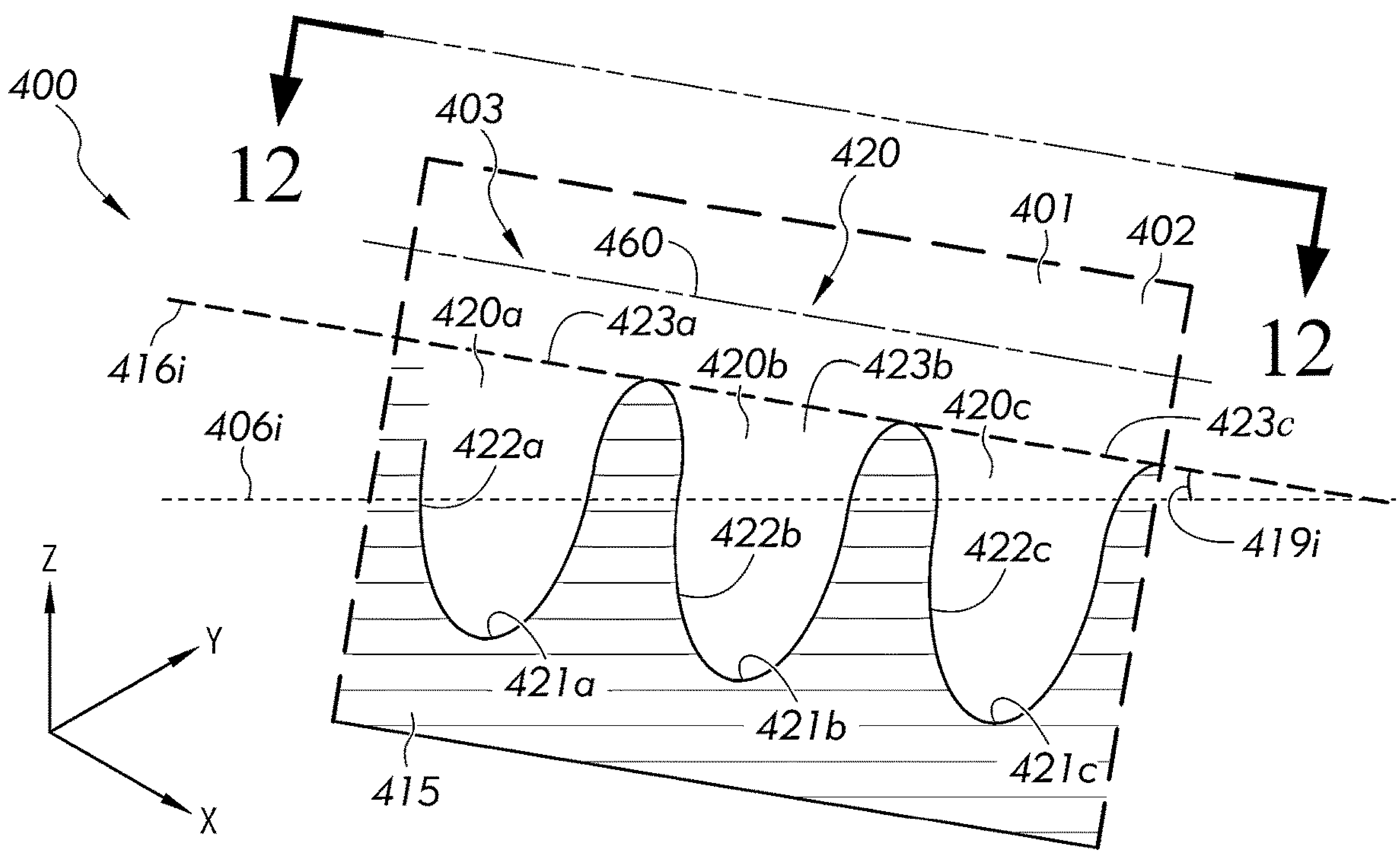
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FIG. 11

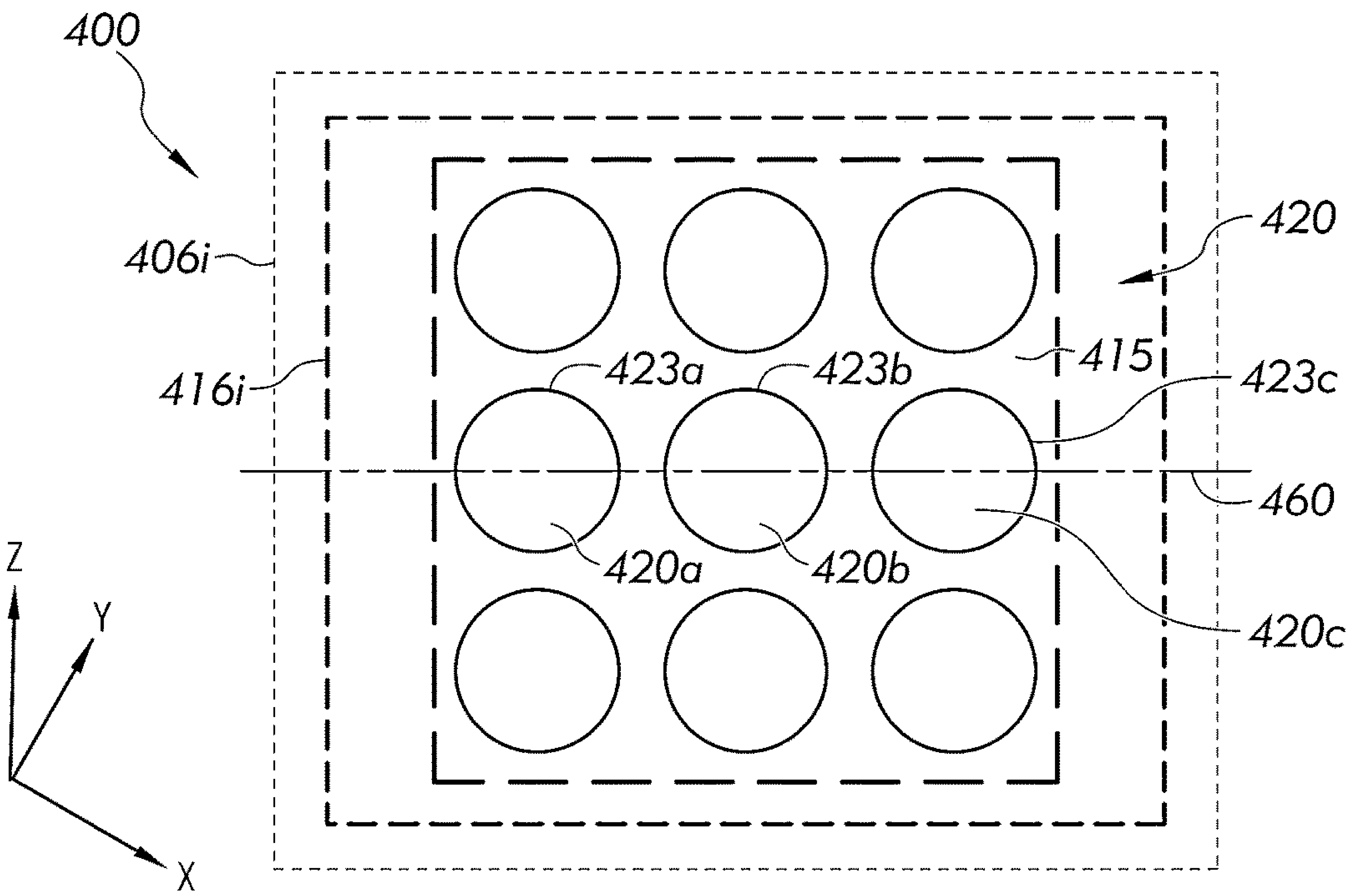
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FIG. 12

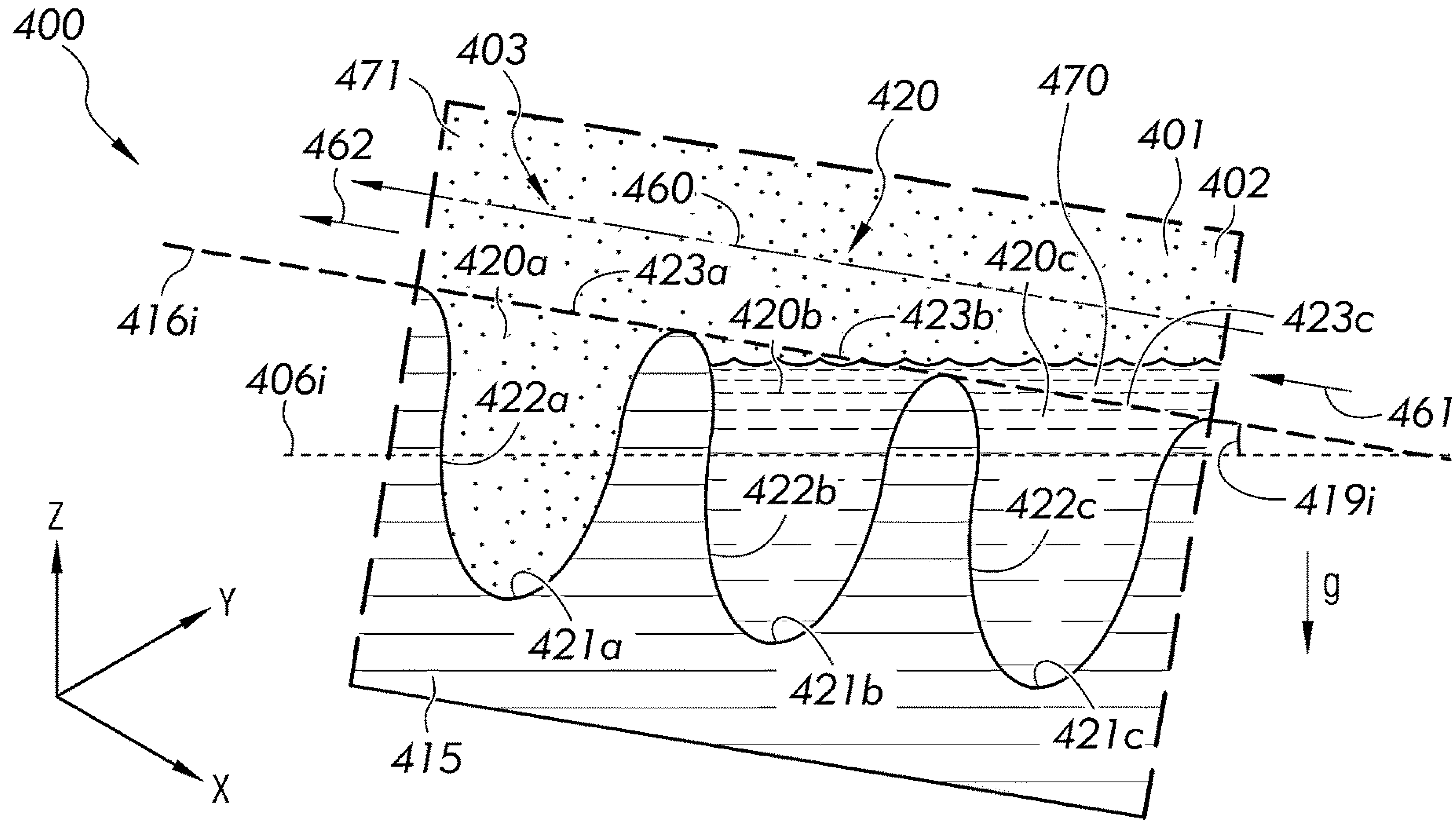
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FIG. 13

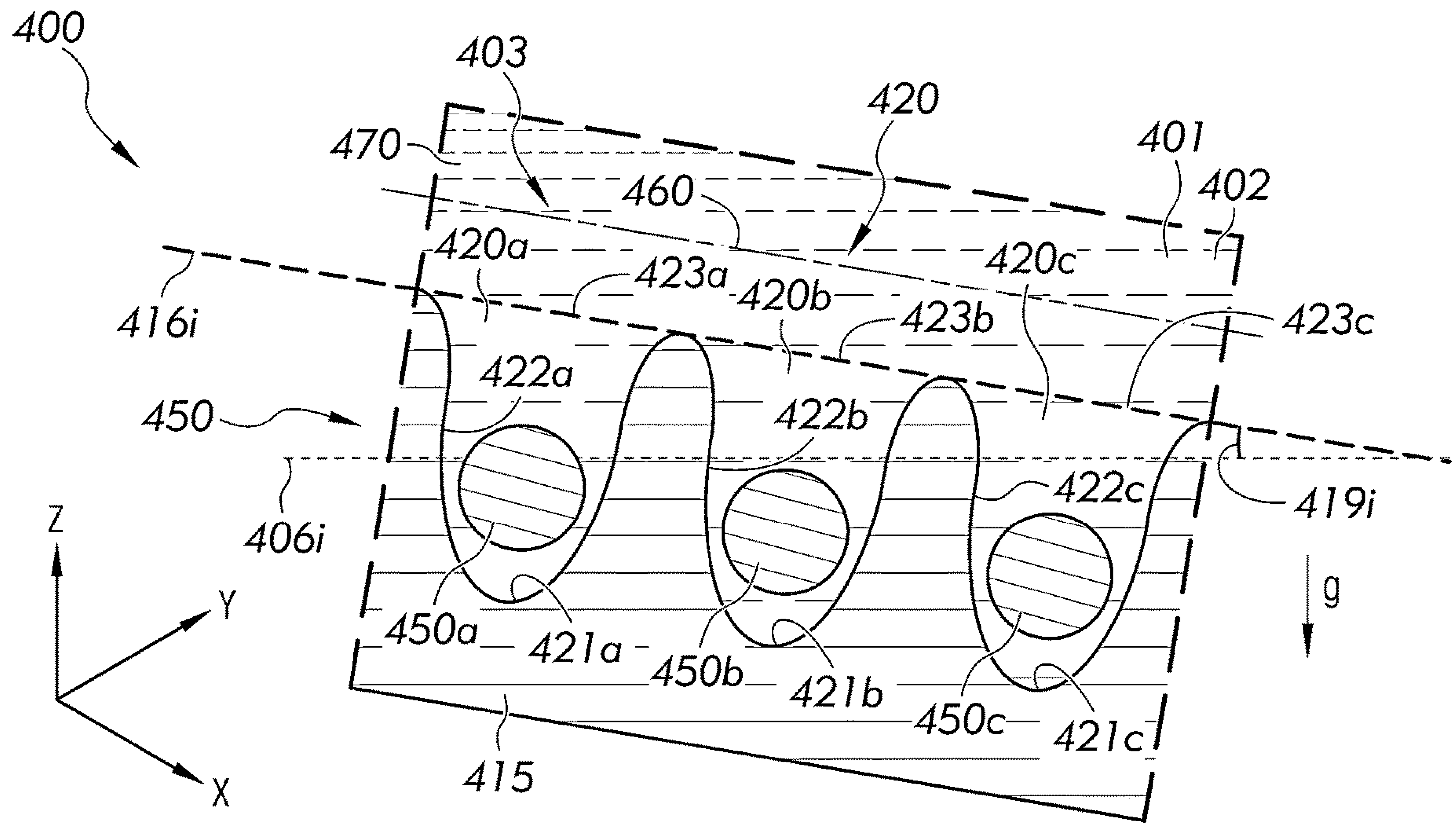
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FIG. 14

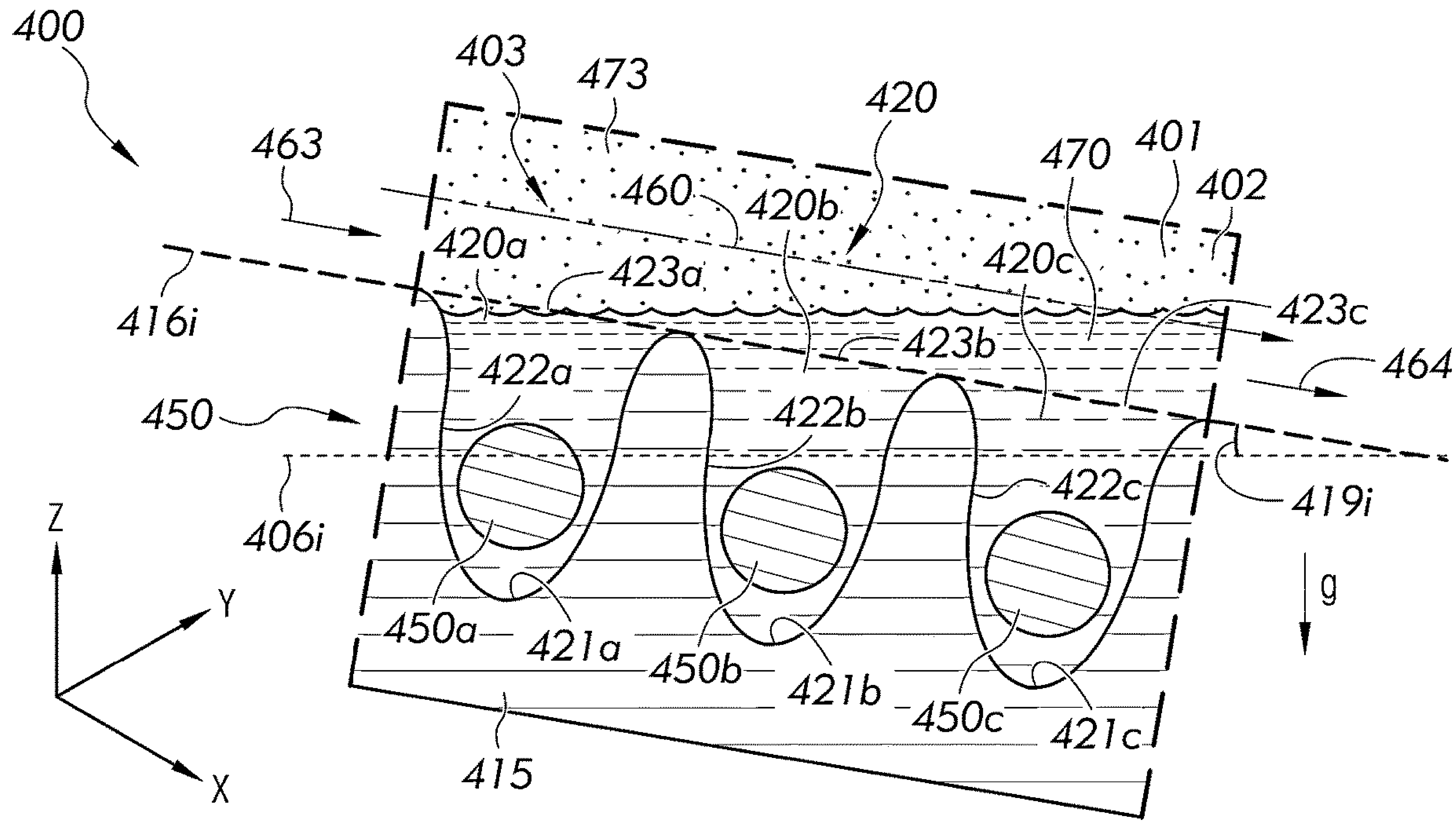
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FIG. 15

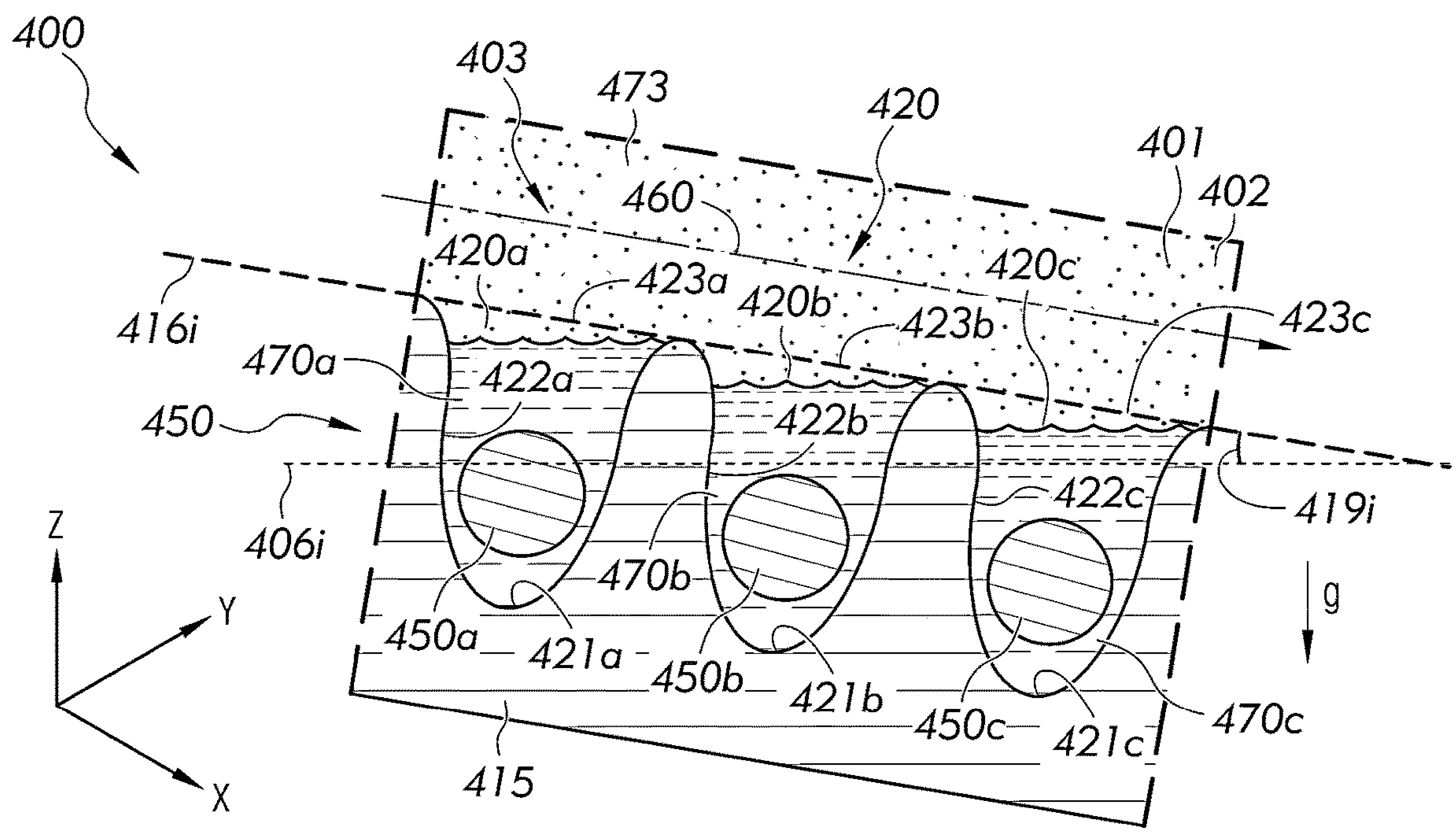
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FIG. 16

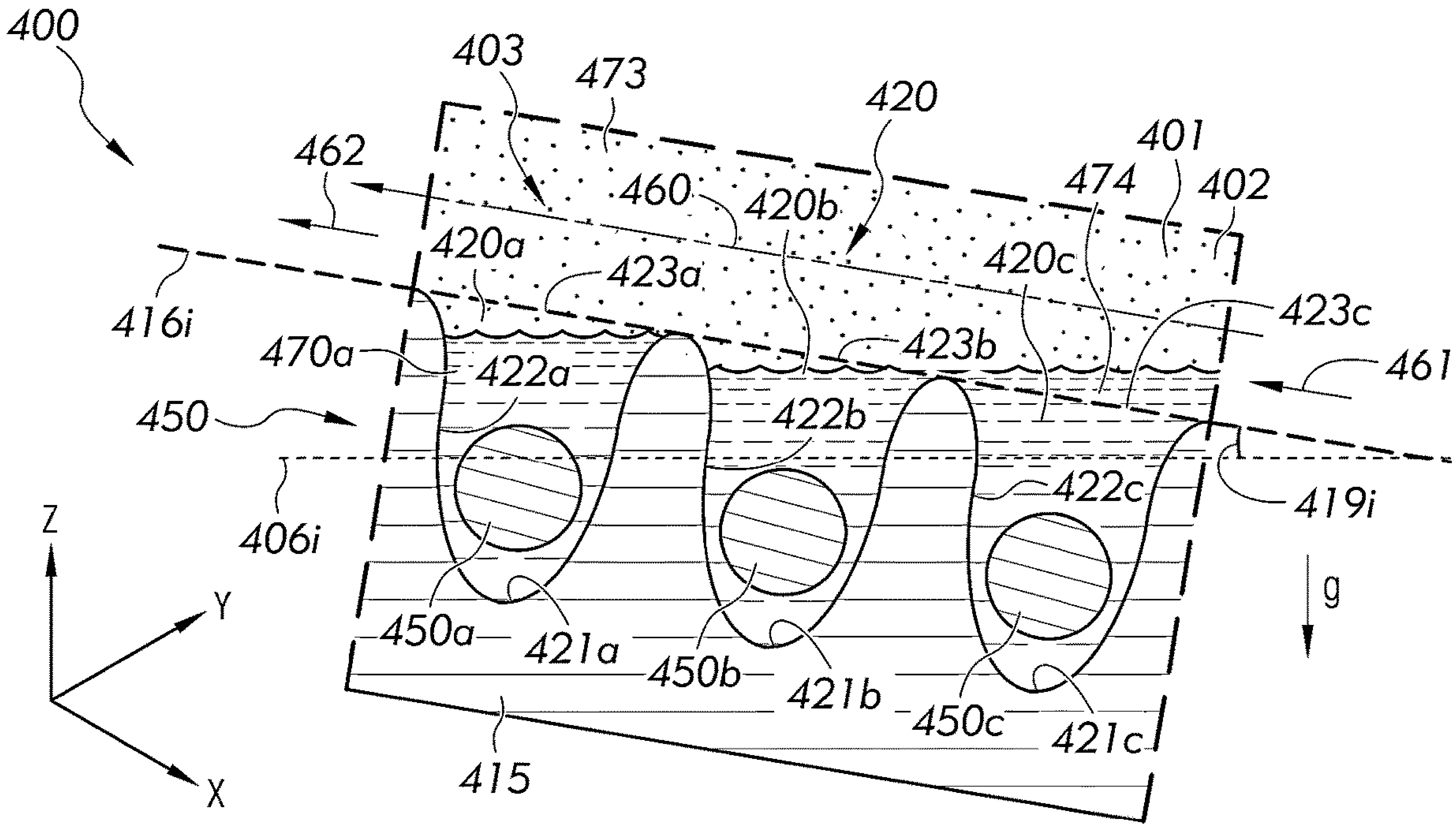
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FIG. 17

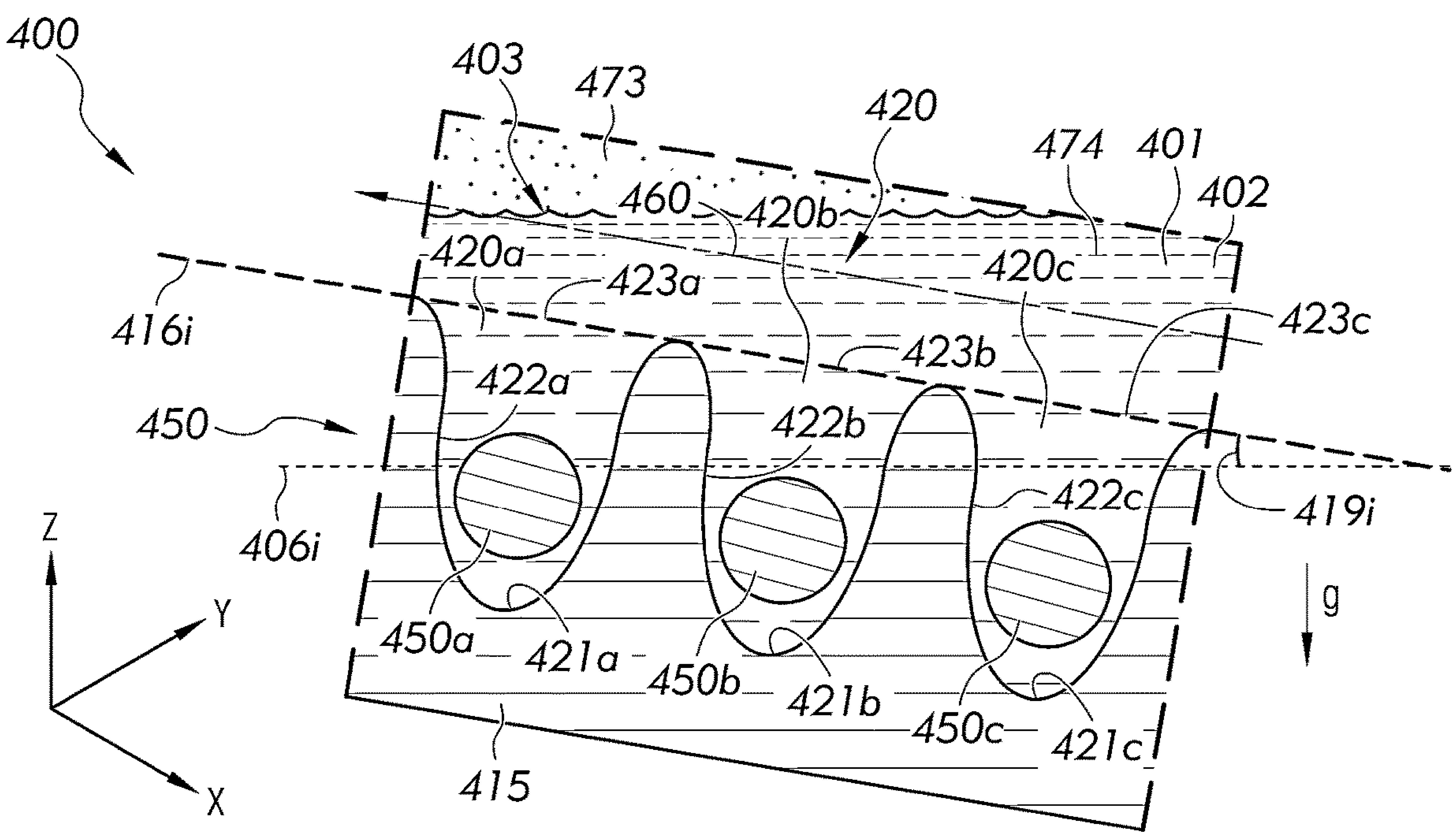
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FIG. 18

3D CELL CULTURE VESSELS FOR MANUAL OR AUTOMATIC MEDIA EXCHANGE

CROSS REFERENCE TO RELATED APPLICATION

This is a continuation application of U.S. patent application Ser. No. 16/628,375 filed on Jul. 13, 2018 (now U.S. Pat. No. 11,345,880, granted May 5, 2022), which is a national stage application under 35 U.S.C. § 371 of International Application No. PCT/US2018/042159 filed on Jul. 13, 2018, which claims the benefit of priority of U.S. Provisional Application Ser. No. 62/532,639 filed on Jul. 14, 2017, entitled "Cell Culture Containers and Methods of Culturing Cells", the content of which are relied upon and incorporated herein by reference in their entireties.

FIELD

The present disclosure relates generally to a cell culture vessel and methods of culturing cells, and more particularly, to a cell culture vessel for containing and basing three-dimensional cell culture, including exchanging media, and methods of culturing three-dimensional cells and methods of exchanging media using the cell culture vessel.

BACKGROUND

It is known to contain three-dimensional cells in a cell culture vessel. It is also known to culture three-dimensional cells in a cell culture vessel.

SUMMARY

The following presents a simplified summary of the disclosure in order to provide a basic understanding of some exemplary embodiments described in the detailed description.

A cell culture vessel (e.g., flask) can provide a sterile microcavity for culturing cells. In some embodiments, culturing cells can provide information related to the study of diseases and toxicology, the efficacy of medications and treatments, characteristics of tumors, organisms, genetics, and other scientific, biological, and chemical principles of and relating to cells. The cell culture vessel provides a sterile, liquid-impermeable microcavity to contain cells during culture.

The microcavity or cell growth chamber can include a bottom surface, a top surface and sidewalls having surfaces. At least one of these surfaces can be adapted for cell growth. For example, to base the culture of spheroid cells, the cell growth surface can include a plurality of microcavities (e.g., micron-sized wells, submillimeter-sized wells) arranged, for example, in an array. The cell growth surface can be integral to the flask or can be a separate substrate placed or affixed in the cell growth chamber. The top surface, the bottom surface, one or more side surfaces or a combination of these can include microcavities in an array. Microcavities can be, for example, formed in an undulating or sinusoidal shape forming microcavities or microwells having rounded tops and rounded bottoms. In some embodiments, the flask can be filled with a material (e.g., media, solid, liquid, gas) that facilitates growth of three-dimensional cell cultures (e.g., cell aggregates, spheroids). For example, a media including cells suspended in a liquid can be added to the cell culture chamber. The suspended cells can collect in the plurality of microcavities and can form (e.g., grow) into grouping or

cluster of cells. The grouped or clustered cells grow in three dimensions to form cells in 3D, otherwise known as a spheroid or an organoid. A single cluster of cells or spheroid forms in a single microcavity. Thus, a cell culture chamber, having a cell culture surface having an array of microcavities, can be used to culture an array of spheroids, each residing in its own microcavity.

For example, in some embodiments, a single spheroid can form in each microcavity of the plurality of microcavities. Cells will settle into a microcavity by gravity. One or more cells suspended in liquid media will fall through the liquid and settle within each microcavity. The shape of the microcavity (e.g., a concave surface defining a well), and a surface coating of the microcavity that prevents the cells from attaching to the surface can also facilitate growth of cells into three-dimensional form, forming a spheroid in each microcavity.

During culturing, the spheroids can consume media (e.g., food, nutrients) and produce metabolites (e.g., waste) as a byproduct. Thus, in some embodiments food in the form of media can be added to the cell culture chamber during culturing and waste media can be removed from the cell culture chamber during culturing. This ability to change the media to feed cells and remove waste products, promotes the long-term culture of cells. However, adding and removing media may displace spheroids resting in microcavities. This is especially true when the microcavities are coated with a low binding coating to prevent the cells from sticking to the microcavity surface. In this disclosure, structures are disclosed which reduce the risk of displacing spheroids from the microcavities, thus promoting the long-term culture of spheroids. As compared to two-dimensional cell cultures, in some embodiments, three-dimensional cell cultures can produce multicellular structures that are more physiologically accurate and that more realistically represent an environment in which cells can exist and grow in real life applications as compared to simulated conditions in a laboratory. For example, three-dimensional cell cultures have been found to more closely provide a realistic environment simulating "in vivo" (i.e. within the living, in a real-life setting) cell growth; whereas two-dimensional cell-cultures have been found to provide an environment simulating "in vitro" (i.e., within the glass, in a laboratory setting) cell growth that is less representative of a real-life environment occurring outside of a laboratory. By interacting with and observing the properties and behavior of three-dimensional cell cultures, advancements in the understanding of cells relating to, for example, the study of diseases and toxicology, the efficacy of medications and treatments, characteristics of tumors, organisms, genetics, and other scientific, biological, and chemical principles of and relating to cells can be achieved.

In some embodiments, it is desirable to culture large numbers of spheroids for long periods of time in order to amplify signals during the study of diseases and toxicology, the efficacy of medications and treatments, characteristics of tumors, organisms, genetics and other scientific, biological, and chemical principles of and relating to cells. For example, if it is desired to test for metabolites of a drug, it may be necessary to culture cells for a long period of time in order give the cultured cells time enough to metabolize the drug in sufficient quantity for the metabolite to be measurable. In some embodiments, it is also desirable to grow large numbers of spheroids in as small a physical space as possible, to economize space in incubators during cell culture. Single layer cell culture devices, including flasks, are known in the art. Multilayer cell culture devices, which allow a user to culture more cells per cubic inch of incubator space are also known; see, for example, U.S. Pat. Nos. 7,745,209, 8,273,572, 8,470,589, 8,846,399, 9,045,721, 9,845,451 and 8,178,345, all assigned to Corning Incorporated. FIG. 1 is taken from U.S. Pat. No. 8,178,345 and is provided as an illustration of the prior art. Multi-layer devices structured and arranged to grow spheroid cells have been disclosed (see, for example, WO2016/069885 and WO2016/069892, also assigned to Corning Incorporated). All cited references are herein incorporated by reference. The present disclosure provides a single-layer or multi-layer cell culture vessel adapted to reduce the risk of displacing spheroids, resting in microcavities, during media changes.

In embodiments, a cell culture vessel includes a base defining a base plane extending in a first direction and a second direction perpendicular to the first direction. In embodiments, the cell culture vessel has a plurality of cell culture chambers stacked one atop another, each cell culture chamber having a top, a bottom and sidewalls to form a cell culture chamber. Each of the top, bottom and sidewalls have an interior surface facing the inside of the cell culture chamber. In embodiments, at least the bottom surface has an array of microcavities for basing the culture of cells as spheroids. In embodiments, each bottom surface of each cell culture chamber is at an angle with respect to the base plane. Further, liquid can flow through into each cell culture chamber via an inlet and out of each cell culture chamber via an outlet. The angled cell culture surface prevents spheroids from being dislodged during media changes.

In embodiments, a cell culture vessel can include one or more cell culture chambers. Each cell culture chamber (defined by a top, sidewalls and a bottom) has at least a bottom surface that has an array of microcavities to allow cells to grow in 3D or spheroid formation in the cell culture chambers. In addition, the vessel has a table plane, perpendicular to the direction of gravity, the plane extending in a first direction, and a second direction perpendicular to the first direction. The table plane is parallel to the plane of the surface upon which the vessel sits. The table plane is, for example the flat plane of a table or workspace upon which the vessel sits. The bottom surface of each cell culture chamber can be oriented at a first angle relative to the first direction of the table plane, and at a second angle relative to the second direction of the table plane. In embodiments, an absolute value of at least one of the first angle and the second angle can be greater than zero. In some embodiments, a method can include culturing cells in the cell culture vessel.

In some embodiments, a method of culturing cells can include introducing liquid into a plurality of cell culture chambers while a base plane of the vessel is oriented perpendicular relative to the direction of gravity. The base plane can extend in a first direction and a second direction perpendicular to the first direction. The method can include culturing cells in the at least one cell culture chamber after introducing the liquid into the at least one chamber, and flowing liquid in a flow direction parallel to the substrate plane while culturing the cells in the at least one microcavity.

The above embodiments are exemplary and can be provided alone or in any combination with any one or more embodiments provided herein without departing from the scope of the disclosure. Moreover, it is to be understood that both the foregoing general description and the following detailed description present embodiments of the present disclosure, and are intended to provide an overview or framework for understanding the nature and character of the embodiments as they are described and claimed. The accompanying drawings are included to provide a further understanding of the embodiments, and are incorporated into and constitute a part of this specification. The drawings illustrate various embodiments of the disclosure, and together with the description, serve to explain the principles and operations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, embodiments, and advantages of the present disclosure can be further understood when read with reference to the accompanying drawings in which:

FIG. **1** represents the PRIOR ART and shows a cut-away view of a multilayer cell culture vessel.

FIG. **2** is a schematic diagram of a perfusion system, in an embodiment of the present disclosure.

FIG. **3** is a schematic cross-section of a multilayer cell culture vessel in an embodiment of the present disclosure.

FIG. **4** schematically illustrates a plan view of an embodiment of a cell culture vessel including a base plane and a table plane in accordance with embodiments of the disclosure;

FIG. **5** shows a front view of an embodiment of the cell culture vessel including a base plane and a table plane along line **5-5** of FIG. **4** in accordance with embodiments of the disclosure;

FIG. **6** shows a side view of an embodiment of the cell culture vessel including a base plane and a table plane along line **506** of FIG. **4** in accordance with embodiments of the disclosure;

FIG. **7** shows an exemplary embodiment of a cross-sectional view of the cell culture vessel including a base plane and a table plane along line **7-7** of FIG. **4** in accordance with embodiments of the disclosure;

FIG. **8** shows an alternative exemplary embodiment of a portion of the cell culture vessel taken at view **8** of FIG. **7** including a base including a thicker portion and a thinner portion in accordance with embodiments of the disclosure;

FIG. **9** shows an alternative exemplary embodiment of a portion of the cell culture vessel taken at view **8** of FIG. **7** including a base including a base in accordance with embodiments of the disclosure;

FIG. **10** shows a bottom view of the cell culture vessel including a base plane and a table plane along line **10-10** of FIG. **5** in accordance with embodiments of the disclosure;

FIG. **11** illustrates an enlarged schematic representation of an exemplary embodiment of a portion of the cell culture vessel taken at view **55** of FIG. **7** including a substrate including a plurality of cell culture chambers in accordance with embodiments of the disclosure;

FIG. **12** shows a plan view of the portion of the cell culture vessel including a cell culture surface including the plurality of microcavities along line **12-12** of FIG. **11** in accordance with embodiments of the disclosure;

FIG. **13** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of flowing a liquid along a flow path in accordance with embodiments of the disclosure;

FIG. **14** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of culturing cells in at least one microcavity of a plurality of microcavities in a bottom surface of a cell culture chamber in accordance with embodiments of the disclosure;

FIG. **15** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of culturing cells in at least one microcavity of a plurality of microcavities in a bottom surface of a cell culture chamber while flowing a liquid and a gas along a flow path in accordance with embodiments of the disclosure;

FIG. **16** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of culturing cells in at least one microcavity of a plurality of microcavities of a bottom surface of a cell culture chamber in accordance with embodiments of the disclosure;

FIG. **17** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of culturing cells in at least one microcavity of a plurality of microcavities of a substrate while flowing a liquid and a gas along a flow path in accordance with embodiments of the disclosure; and

FIG. **18** shows an alternative exemplary embodiment of the portion of the cell culture vessel of FIG. **11** including a method of culturing cells in at least one microcavity of a plurality of microcavities of a substrate in accordance with embodiments of the disclosure.

DETAILED DESCRIPTION

Features will now be described more fully hereinafter with reference to the accompanying drawings in which exemplary embodiments of the disclosure are shown. Whenever possible, the same reference numerals are used throughout the drawings to refer to the same or like parts. However, this disclosure can be embodied in many different forms and should not be construed as limited to the embodiments set forth herein.

FIG. **1** represents the PRIOR ART and shows a cut-away view of a multilayer cell culture vessel **100**. As illustrated in this PRIOR ART device, a multilayer cell culture vessel is illustrated having a top surface **102** a bottom surface **104**, a necked opening **110** with a cap **112**, and sidewalls **120**. There are a plurality of cell culture chambers **114**. The bottom surface **116** of the cell culture chambers **114** are made up of gas permeable, liquid impermeable material which is adjacent to a tracheal space **124**. To fill this vessel, a use introduces fluid into the necked opening, distributing liquid into each cell culture chamber via the manifold **108**. Perfusion is not possible in this vessel.

FIG. **2** is a schematic diagram of a perfusion system, in an embodiment of the present disclosure. FIG. **2** illustrates a multilayer cell culture vessel **400**. The vessel **400** has a top surface **102** and a bottom surface **482**. Each layer of the multilayer cell culture vessel is a discrete cell culture chamber **200**. Each cell culture chamber has a bottom **201**, a top **202**, and sidewalls **203** (not shown in FIG. **2**) that together define the cell culture chamber **200**. Each of the bottom **201**, top **202** and sidewalls **203** have an interior surface that face into the cell chamber **200**. In embodiments, there are at least two cell culture chambers stacked one above, each having a bottom, a top and sidewalls. In embodiments, at least one of these surfaces has microcavities **420**. In embodiments, at least the bottom surface has microcavities **420**. In embodiments the microcavities **420** are provided in an array of microcavities. FIG. **2** illustrates an embodiment having five layers of cell culture chambers **200**, each cell culture chamber **200** having a bottom **201** having an array of microcavities **420**. As used herein, "array" means merely a plurality of microcavities **420**.

These microcavities **420** are structured and arranged to provide base for cultured cells to form three dimensional structures, or spheroids. That is, the microcavities **420** are coated with a non-binding coating to keep cells from sticking to the substrate and forming monolayers of cells, and in addition, these microcavities have an appropriate geometry to encourage cells to cluster together and form spheroids in culture. In an embodiment, and as shown in FIGS. **11** and **13-18**, these microcavities **420** are sinusoidal in shape. That is, the microcavities **420** have rounded tops and rounded bottoms. However, microcavities **420** can be in any shape or geometry consistent with the formation of spheroids in culture.

FIG. **3** is a schematic cross-section of a multilayer cell culture vessel **400** in an embodiment of the present disclosure. FIG. **3** illustrates an inlet **210** at the top of the vessel along one side of the vessel and an outlet **215** at the bottom of the vessel along another side of the vessel. In embodiments, the outlet **215** is on the opposite side of the vessel from the inlet **210**. Inlet **210** is in fluid communication with each cell culture chamber **200** through an inlet manifold **21**. Outlet **216** is in fluid communication with each cell culture chamber **200** through an outlet manifold **217**. Each cell culture chamber has an inlet opening **218** between the cell culture chamber **200** and the inlet manifold **216** and an outlet opening **219** between the cell culture chamber **200** and the outlet manifold **217**. In embodiments, and as shown in FIG. **3**, no pump is necessary to flow liquid through the vessel and fluid flows by the force of gravity, flowing across the bottom surface of a cell culture chamber that is at an angle compared to a table plane **406**.

Referring back to FIG. **2**, liquid (such as media) can flow into the inlet **210**, through the inlet manifold **216**, into each cell culture chamber **200** through the inlet opening **218**, out of the cell culture chamber **200** through the outlet opening **219**, through the outlet manifold **217**, and exit the vessel through the outlet **215**. In embodiments, the flow of liquid may be accomplished by means of a pump **209**, or by gravity. In embodiments, a liquid reservoir is provided both to provide liquid to the inlet **210** (**213**) and to collect liquid from the outlet **215** (**211**). In embodiments, a valve **212** may be present to control flow from a fresh media reservoir **213** and to a waste or collection reservoir **211**. In embodiments, tubing may connect inlet to a pump (not shown) to allow liquid to be pumped into the inlet **210**.

In embodiments, liquid can fill the vessel **400** from the outlet **215**. For example, liquid can enter the outlet **215**, by being pumped into the vessel **400** by the pump **209**. When filling the vessel **400** in this way, liquid can enter the vessel through the inlet, and flow into the outlet manifold **217**, can enter each cell culture chamber **200** through the outlet openings **219**, fill each cell culture chamber **200**, then fill the inlet manifold through the inlet openings **218**. This allows air to exit the vessel through the inlet **210** as the vessel is filling. Filling the device in this manner may assist with evacuation of air out of the vessel and reduce the formation of bubbles during the filling process.

FIG. **3** also illustrates a multilayer cell culture vessel having internal tracheal spaces **204** adjacent to the bottom **201** of each cell culture chamber **200**. In embodiments, the bottom **201** of each cell culture chamber is made from gas permeable, liquid impermeable material. Using this material increases gas exchange from the tracheal or gas spaces **220** through the gas permeable liquid impermeable material into the microcavities **420** which contain spheroids. This gas permeable material makes the vessel more versatile. For example, if used in a non-perfusion mode, cells have access to oxygen in the absence of perfused media. However, if the perfusion system is in operation, oxygen can be delivered to the cells dissolved in the circulating media. In this way, the vessel can be used both as a perfusion system and as a static system. However, in a perfusion system, this gas permeable material may not be necessary as oxygen is provided to the spheroids via the oxygenated media circulating through the cell culture chambers **200**.

As shown in FIG. **3**, the vessel has a base **405** that is angled. That is, the base **405** is thicker at the inlet side of the vessel **405***a* than at the outlet side of the vessel **405***b*. Because the base is thicker at the inlet side **405***a* than the outlet side **405***b*, the plane of the base (the base plane **407**) is different from the plane of a table **406**. The table plane **406** is intended to be a parallel to a plane perpendicular to gravity representing a table top or incubator shelf. The table plane **406** is parallel to the surface upon which the vessel **400** rests. The stacked cell culture chambers **200** are stacked on the base **405** and are parallel to the base plane **407**. Therefore, each of the stacked cell culture chambers **200** are at an angle (see **417***i* of FIG. **5**, for example) compared to the table plane **406**.

In addition, if the cell culture vessel **400** is square or rectangular, the inlet may be on diagonal corners of the vessel (as shown in FIG. **5**, for example). In that case, it may be that the base **405** has an angle along one plane of the vessel **400**, or the base **405** may have an angle along two planes of the vessel **400**. That is, the base may have an angle along an "X" axis (an X-Z angle, see, for example, **417***i* of FIG. **5**), along a "Y" axis (a Y-Z axis, see, for example, angle **418***i* of FIG. **6**), or along both an "X" and a "Y" axis. That is, the cell culture chambers may be oriented to direct liquid to the outlet wall **480** of the vessel **400** (see FIG. **5**) or the cell culture chambers may be oriented to direct liquid to the outlet corner **481** of the vessel (see, for example, FIG. **4**). Or, stated another way, an absolute value of at least one of the first angle and the second angle is greater than zero.

The base **405** defines a base plane **407** extending in a first direction "X" and a second direction "Y" perpendicular to the first direction. Each bottom surface **201** of each cell culture chamber **200** is parallel to the base plane **407**. In embodiments, the bottom surface **201** of each cell culture chamber **200** is at a first angle relative to the first direction "X" (see **417***i* of FIG. **5**) of the base plane **405** and a second angle relative to the second direction "Y" (see **418***i* of FIG. **6**) of the table plane; and an absolute value of at least one of the first angle and the second angle is greater than zero. As FIG. **3** is a cross-sectional drawing, the second angle is not shown in FIG. **3**, but see FIGS. **4-18** below.

A three-dimensional Cartesian coordinate system, including a first direction "X", a second direction "Y", and a third direction "Z" (with each direction being perpendicular to the other) is provided in FIGS. **3-18** to indicate a particular spatial orientation of the vessel **400** relative to the first direction "X", the second direction "Y", and the third direction "Z". Unless otherwise noted, it is to be understood, however, that the particular spatial orientation of the vessel **400** illustrated in the drawings figures provides an exemplary orientation that is not intended to limit the scope of the features of the vessel **400**. Therefore, in some embodiments, one or more features of the vessel **400** can be provided at a different orientation than the particular orientation depicted in the drawings figures, without departing from the scope of the disclosure. In general, the third direction "Z" indicates the direction parallel with the direction of gravity.

For example, FIG. **4** schematically illustrates a plan view (top down) of an embodiment of the cell culture vessel **400**, FIG. **5** shows a front view of the vessel **400** along line **5-5** of FIG. **4**, and FIG. **6** shows a side view of the vessel **400** along line **6-6** of FIG. **4**. In the drawing figures, the vessel **400** is illustrated as being manufactured from a clear (e.g., transparent) material; although, in some embodiments, the vessel **400** can, alternatively, be manufactured from one or more of a semi-transparent, semi-opaque, or opaque material without departing from the scope of the disclosure. While the embodiments shown in FIGS. **4-18** illustrate a single layer cell culture vessel, for ease of illustration. However, it is to be understood that the device can be in a multilayer configuration, as shown in FIG. **2** and FIG. **3**. In the embodiments shown in FIGS. **4-18**, the vessel wall **401** forms the bottom **482**, top **202**, and sidewalls **203** of the vessel.

As shown in FIG. **4**, in some embodiments, the cell culture vessel **400** can include a base **405** having a bottom surface **482** parallel with a table plane **406** extending in the first direction "X" and the second direction "Y", where the first direction "X" and the second direction "Y" are perpendicular. Additionally, the vessel **400** can include a first aperture **432** (shown as inlet **210** in FIG. **2**) or second aperture **431** (shown at the top of FIG. **4**—FIG. **4** is a top-down view—and shown as outlet **215** in FIG. **2**) extending through the wall **401** in fluid communication with the cell culture chamber **403**, and a second aperture, or inlet **432** extending through the wall in fluid communication with the cell culture chamber **403**. FIG. **4** illustrates shows the vessel having a top **202** and sidewalls **203**. The sidewall from which the second aperture **431** extends is the outlet side **480** of the vessel **400**. The corner from which the second aperture **431** extends is the outlet corner **481** of the vessel.

As shown in FIG. **5** and FIG. **6**, in some embodiments, the second aperture **432** can be spaced from the first aperture **431** along an outward direction (e.g., the third direction "Z") extending away from the table plane **406** and perpendicular to the table plane **406**, as represented by dimension "dz". Additionally, as shown in FIG. **4** and FIG. **5**, in some embodiments, the first aperture **431** can be spaced from the second aperture **432** in the first direction "X", as represented by dimension "dx". Likewise, as shown in FIG. **4** and FIG. **6**, in some embodiments, the first aperture **431** can be spaced from the second aperture **432** in the second direction "Y", as represented by dimension "dy". Note the Cartesian coordinates in the figures to assist with this description. In embodiments, the first aperture **431** and second aperture **432** inlet and outlet are on opposite walls of the vessel **400**. The wall from which the first aperture **431** (the outlet) extends is the outlet wall **480**. The corner containing the first aperture **431** (the outlet) is the outlet corner **481**. FIGS. **5** and **6** show the vessel having a top **202**, sidewalls **203** and a bottom **405** defining a cell culture chamber **403**.

As shown in FIG. **11**, which shows an enlarged view taken at view **11** of FIG. **7**, and as shown in FIG. **12**, which shows a partial view along line **12-12** of FIG. **11**, in some embodiments, the vessel **400** can include an array of microcavities **420** in the bottom surface or substrate **415** of the cell culture chamber **403**. In some embodiments, the microcavities **420** are formed in a substrate **415** that is placed into or attached to the cell culture chamber. In other embodiments, the microcavities **420** are formed directly into the bottom surface of the cell culture chamber or multiple chambers. Whether formed in a substrate or directly in the bottom surface of the cell culture chamber(s), the bottom surface **415** of the cell culture chamber can include a plurality of microcavities **420**, and each microcavity **420***a*, **420***b*, **420***c* of the plurality of microcavities **420** can include a bottom rounded concave surface **421***a*, **421***b*, **421***c* defining the well of the microcavities **422***a*, **422***b*, **422***c* and an opening **423***a*, **423***b*, **423***c* defining a path into the well **422***a*, **422***b*, **422***c*. The bottom surface of the cell culture chamber or substrate

415 can define a substrate plane 416*i*, parallel with the base plane 407, and at an angle with respect to the table plane 406*i*, on which the opening 423*a*, 423*b*, 423*c* of each microcavity 420*a*, 420*b*, 420*c* of the plurality of microcavities 420 is located. For clarity purposes, the substrate 415 is not shown in, for example, FIGS. 4-18. Rather, the substrate plane 416*i*, parallel with the base plane 407, and at an angle with respect to the table plane 406*i*, is schematically illustrated in FIGS. 4-18 with the understanding that the bottom surface of the cell culture chamber, or substrate plane 416*i*, represents a plane on which the opening 423*a*, 423*b*, 423*c* of each microcavity 420*a*, 420*b*, 420*c* of the plurality of microcavities 420 of the substrate 415 is located, schematically represents the existence of the cell culture surface, or substrate 415 in the cell culture chamber 403.

As shown in FIG. 7, in some embodiments, the vessel 400 can include a plurality of cell culture chambers 200, defining a corresponding plurality of substrate planes 416*a*, 416*b*, 416*c*, 416*d*. Although four substrate planes 416*a*, 416*b*, 416*c*, 416*d* are provided, it is to be understood that, in some embodiments, one substrate plane (e.g., substrate plane 416*i*) can be provided, or more than one (e.g., two, three . . . seven, eight, ten, etc.) substrate planes can be provided without departing from the scope of the disclosure. Likewise, for clarity purposes, relative to the substrate planes 416*a*, 416*b*, 416*c*, 416*d*, a corresponding plurality of table planes 406*a*, 406*b*, 406*c*, 406*d*, are shown to schematically provide an illustrative representation of an orientation of the substrate planes 416*a*, 416*b*, 416*c*, 416*d* relative to the table plane 406. That is, in some embodiments, each of the substrate planes 416*a*, 416*b*, 416*c*, 416*d*, can intersect the table plane 406. Thus, the corresponding table planes 406*a*, 406*b*, 406*c*, 406*d*, which are parallel to the table plane 406, provide a schematic illustration of the orientation of each substrate plane 416*a*, 416*b*, 416*c*, 416*d*, relative to the table plane 406. Thus, in some embodiments, substrate plane 416*i* is shown with the understanding that the substrate plane 416*i* can be representative of and include features of any one or more of the plurality of substrate planes 416*a*, 416*b*, 416*c*, 416*d*, the substrate being the cell culture surface having an array of microcavities. Likewise, in some embodiments, table plane 406*i* is shown with the understanding that the table plane 406*i* can be representative of a corresponding plane parallel to the table plane 406 provided for purposes of clarity to schematically illustrate a respective orientation of the substrate plane 416*i* relative to the table plane 406.

Moreover, in some embodiments, a respective first angle 417*i* (on the "XY" plane), a respective second angle 418*i* (the "YZ" plane), and a respective plane angle 419*i* (which may include a first angle 417*i*, a second angle 418*i* or both) can be provided to an orientation of the base plane or substrate plane 416*i* relative to the table plane 406, as represented by a corresponding table plane 406*i*. For example, as shown in FIG. 5, in some embodiments, the base plane 416*i* can be oriented at a first angle 417*i* relative to the first direction "X". Likewise, as shown in FIG. 6, in some embodiments, the base plane 416*i* can be oriented at a second angle 418*i* relative to the second direction "Y". In some embodiments, an absolute value of at least one of the first angle 417*i* and the second angle 418*i* can be greater than zero. For example, in some embodiments an absolute value of the first angle 417*i* can be greater than zero, and an absolute value of the second angle 418*i* can be greater than zero. As shown in FIG. 7, in some embodiments, an absolute value of a base plane angle 419*a*, 419*b*, 419*c*, 419*d* between the table plane 406*a*, 406*b*, 406*c*, 406*d* and the substrate plane 416*a*, 416*b*, 416*c*, 416*d* can be from about 1° to about 30°. For example, in some embodiments, based at least on the orientation of the base plane 416*i* relative to the table plane 406 (e.g., relative to the first direction "X" defining the first angle 417*i*, and relative to the second direction "Y" defining the second angle 418*i*) an absolute value of the plane angle 419*a*, 419*b*, 419*c*, 419*d* can be from about 1° to about 30°. For example, in some embodiments, an absolute value of the plane angle 419*a*, 419*b*, 419*c*, 419*d* can be about 5°.

FIG. 8 and FIG. 9 show exemplary embodiments of a partial cross-section view of a vessel 400 taken at view 8 of FIG. 7. For example, as shown in FIG. 8, in some embodiments, the base 405 defining the base plane 407 can include a thicker portion 405*a* of the base 405 relative to a thinner portion 405*b* of the base 405. In addition or alternatively, as shown in FIG. 9, in some embodiments, the vessel 400 can include a step 405*c* positioned relative to the base 405 to elevate at least a portion of the vessel 400. The step 405*c* and the base 405 can define the base plane 407. For example, in some embodiments, a step 405*c* can be positioned underneath a corner of the base 405 to elevate the vessel 400 and provide the base 405 of the vessel 400 at an angle relative to a horizontal plane perpendicular to the direction of gravity.

In addition or alternatively, as shown in FIG. 10, which shows a bottom view of the vessel 400 along line 10-10 of FIG. 5, in some embodiments, the table plane 406 can be defined based on three points P1, P2, P3 located on the bottom side of base 405 of the vessel 400. For example, by selecting (e.g., identifying) three points P1, P2, P3 on the bottom side of the base 405, a plane, representing the table plane 406, can be defined. In some embodiments, one or more of the points P1, P2, P3 can include one or more structural features of the base 405, including but not limited to protrusions and bases that can be attached (e.g., integral, bonded) to the base 405 or separate from the base 405.

Turning back to FIG. 8, in some embodiments, the inner surface 402*a* of the base 405 can include an inclined surface 402*a* extending from the thicker portion 405*a* to the thinner portion 405*b*. In some embodiments, the inclined surface 402*a* can be parallel to the table plane 416*i*. Likewise, as shown in FIG. 9, in some embodiments, based at least on the elevation of a portion of the vessel 400, the step 405*c* can orient the inner surface 402 of the base 405 to include an inclined surface 402*a*. In some embodiments, the inclined surface 402*a* can be parallel to the table plane 416*i*. In some embodiments, the first aperture 431 can be positioned at a lower elevation relative to the direction of gravity than the second aperture 432. Thus, in some embodiments, based at least on the force of gravity, liquid within the cell culture chamber 403 can naturally drain toward the first aperture 431. Additionally, in some embodiments, the inclined surface 402*a* of the inner surface 402 of the wall 401 can cause the liquid to naturally drain toward the first aperture 431. In some embodiments, draining the liquid toward the first aperture 431 and out of the vessel 400 can aid in cleaning the cell culture chamber 403, prior to or after culturing cells in the vessel 400.

FIGS. 11-18 show exemplary embodiments of the portion of the vessel 400 including the substrate 415 of FIG. 11 with respect to methods of culturing cells in the cell culture vessel 400. For example, in some embodiments, as shown in FIG. 13, the method can include depositing liquid 470 in at least one microcavity 420*a*, 420*b*, 420*c* of the plurality of microcavities 420. As shown in FIG. 14, in some embodiments, the method can include culturing cells 450 (e.g., spheroid 450*a*, spheroid 450*b*, spheroid 450*c*) in microcavities 420*a*,

420*b*, **420***c* after depositing the liquid **470** in the microcavities **420***a*, **420***b*, **420***c*. In some embodiments, the table plane **406** (e.g., **406***i*) can be perpendicular relative to the direction of gravity "g" while culturing the cells **450** in the at least one microcavity **420***a*, **420***b*, **420***c*.

Additionally, in some embodiments, the method can include flowing material in a flow direction **460** parallel to the substrate plane **416***i* while culturing the cells **450** in the at least one microcavity **420***a*, **420***b*, **420***c*. For example, as shown in FIG. **7**, in some embodiments, as represented by arrow **461**, the method can include passing liquid **470** through the first aperture **431** from outside the vessel **400** into the cell culture chamber **403**, and (as shown in FIG. **13**, by the arrow **461**) flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460** parallel to the substrate plane **416***i*. Likewise, in some embodiments, as shown by arrow **462** in FIG. **13**, the method can include passing gas **471** through the second aperture **432** (as shown in FIG. **7**, by the arrow **462**) from the cell culture chamber **403** to outside the vessel **400** while flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460**. In some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* of the plurality of microcavities **420** while flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460**. For example, in some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* while at least one of passing liquid **470** through the first aperture **431** from outside the vessel **400** into the cell culture chamber **403** (arrow **461**) and passing gas **471** through the second aperture **432** from the cell culture chamber **403** to outside the vessel **400** (arrow **462**).

As shown in FIG. **15**, in some embodiments, the method can include flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460** parallel to the substrate plane **416***i*, and (as represented by arrow **464**) passing the liquid **470** through the first aperture **431** from the cell culture chamber **403** to outside the vessel **400** (as shown in FIG. **7**, by the arrow **464**). Additionally, as shown by arrow **463** the method can include passing gas **473** through the second aperture **432** from outside the vessel **400** into the cell culture chamber **403** (as shown in FIG. **7**, by arrow **463**) while flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460**. As shown in FIG. **16**, in some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* of the plurality of microcavities **420** while flowing the liquid **470** in the cell culture chamber **403** along the flow direction **460**. In some embodiments, based at least on passing the gas **473** into the cell culture chamber **403** (arrow **463**), the liquid **470** can be removed from the cell culture chamber **403** and, in some embodiments, each microcavity **420***a*, **420***b*, **420***c* can include a respective portion of liquid **470***a*, **470***b*, **470***c*. For example, in some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* while at least one of passing gas **473** through the second aperture **432** from outside the vessel **400** into the cell culture chamber **403** (arrow **463**) and passing liquid **470** through the first aperture **431** from the cell culture chamber **403** to outside the vessel **400** (arrow **464**).

As shown in FIG. **17** and FIG. **18**, in some embodiments, the method can include flowing material in a flow direction **460** parallel to the substrate plane **416***i* while culturing the cells **450** in the at least one microcavity **420***a*, **420***b*, **420***c*. For example, as shown in FIG. **7**, in some embodiments, as represented by arrow **461**, the method can include passing liquid **474** (e.g., different liquid as compared to liquid **470**) through the first aperture **431** from outside the vessel **400** into the cell culture chamber **403**, and (as shown in FIG. **61**, by the arrow **461**) flowing the liquid **474** in the cell culture chamber **403** along the flow direction **460** parallel to the substrate plane **416***i*. In some embodiments, the liquid **474** can displace the liquid **470***a*, **470***b*, **470***c* in each microcavity **420***a*, **420***b*, **420***c* with, for example, new liquid **474** that aids in the culturing of the cells **450**. Likewise, in some embodiments, as shown by arrow **462** in FIG. **17**, the method can include passing gas **473** through the second aperture **432** (as shown in FIG. **7**, by the arrow **462**) from the cell culture chamber **403** to outside the vessel **400** while flowing the liquid **474** in the cell culture chamber **403** along the flow direction **460**. In some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* of the plurality of microcavities **420** while flowing the liquid **474** in the cell culture chamber **403** along the flow direction **460**. For example, in some embodiments, the method can include culturing cells **450** in at least one microcavity **420***a*, **420***b*, **420***c* while at least one of passing liquid **474** through the first aperture **431** from outside the vessel **400** into the cell culture chamber **403** (arrow **461**) and passing gas **473** through the second aperture **432** from the cell culture chamber **403** to outside the vessel **400** (arrow **462**).

In some embodiments, the passing of liquid and gas in to or out of the first aperture **431** and the second aperture **432** can be accomplished automatically based on, for example, operation of a system including pumps, reservoirs, tubing, etc. connected to at least one of the first aperture **431** and the second aperture **432** that facilitates automatic passing of liquid and gas into or out of the first aperture **431** and the second aperture **432**. Accordingly, in some embodiments, for example during extended periods of culturing cells **450**, media exchange (e.g., replacement, replenishment, removal) of material (e.g., nutrients and waste) can occur automatically based on operation of the system without, for example, human interaction. Likewise, media exchange can occur without physical manipulation of the vessel **400**. For example, unlike other vessels that can be tilted, inverted, rotated, etc. to exchange media within the cell culture chamber **403** during culturing, the vessel **400** can remain stationary (e.g., without relative movement) during the entire cell culturing process and the media can, nonetheless be exchanged based on the features of the disclosure.

Moreover, providing the substrate plane **416***i* at an angle (e.g., first angle **417***i*, second angle **418***i*, plane angle **419***i*) relative to the table plane **406** can provide several advantages. For example, in some embodiments, providing the substrate **415** at an orientation with an absolute value of the plane angle **419***i* from about 1° to about 30° relative to the table plane **406** when, for example, the table plane **406** is substantially perpendicular relative to the direction of gravity "g" can provide the substrate **415** at an orientation that provides better culturing of the cells **450**. For example, without intending to be bound by theory, it is believed that, in some embodiments, orienting the substrate plane **416***i* with an absolute value of the plane angle **419***i* less than 1° would be insufficient to accommodate the flow of liquid along the flow direction **460** without entrapping gas (e.g., air pockets) between two or more substrates **415** and/or within one or more microcavities **420***a*, **420***b*, **420***c*. Additionally, in some embodiments, in order to flow liquid along the flow direction **460** when the substrate plane **416***i* is oriented with an absolute value of the substrate plane angle **419***i* less than 1°, a velocity of the flow can be increased with negative effects on cells and negative effects on retention of cellular spheroids within the microcavities **420**a, **420**b, **420**c. That is, if the substrate plane angle is less than 1°, less than 5°, or less than 30°, for example, the flow rate necessary to effect efficient replacement of media may create turbulence that might disrupt spheroids residing in microcavities. Thus, providing the substrate **415** at an orientation with an absolute value of the plane angle **419**i from about 1° to about 30° relative to the table plane **406** can permit liquid to gradually enter each microcavity **420**a, **420**b, **420**c thus reducing or preventing the likelihood of gas becoming entrapped within the microcavities **420**a, **420**b, **420**c and reducing or preventing the likelihood of disrupting cells being cultured within the microcavities **420**a, **420**b, **420**c.

That is, in some embodiments, based at least in part of the force of gravity, liquid can naturally flow along the substrate **415** while culturing cells **450** without dislodging or disturbing the culturing of the cells **450**. Alternatively, orienting the substrate plane **416**i with an absolute value of the plane angle **419**i less than 1° could lessen the effect of the force of gravity to cause the liquid to naturally flow in the flow direction **460** along the substrate **415**, in which case, an increased velocity flow that could dislodge or disturb the cells **450** can be employed. Likewise, in some embodiments, orienting the substrate plane **416**i with an absolute value of the plane angle **419**i greater than 30° could cause cells **450** within the microcavities **420**a, **420**b, **420**c to fall out of the microcavities **420**a, **420**b, **420**c (e.g., dislodge, dislocate) during culturing. Accordingly, in some embodiments, orienting the substrate plane **416**i with an absolute value of the plane angle **419**i from about 1° to about 30° (e.g., from about 5° to about 30°, from about 5° to about 25°, from about 5° to about 20°, from about 5° to about 15°, from about 5° to about 10°, from about 10° to about 20°, from about 10° to about 30°, from about 1° to about 10°, from about 1° to about 5°, about 3°, about 5°, about 7°) can provide several advantages that cannot be obtained by cell culture vessels including substrates provided at different orientations outside the range of from about 1° to about 30°.

Throughout the disclosure, the terms "material", "liquid", and "gas" can be used to describe properties of a material employed when, for example, culturing cells in the cell culture vessel. Unless otherwise noted, for purposes of the disclosure, "material" can include fluid material (e.g., liquid or gas). Additionally, material can include a culture solution or media including a liquid including solid particles (e.g., cells) suspended in the liquid. Unless otherwise noted, for purposes of the disclosure, "liquid" can include cleaning or rinsing solutions, aqueous solutions, or other liquid that can be added to or removed from the vessel to, for example, clean the cell culture chamber, sterilize one or more features of the substrate and the vessel, prepare the substrate for cellular growth and other uses of liquid. Additionally, liquid can include a culture solution or media including a liquid including solid particles (e.g., cells) suspended in the liquid. Unless otherwise noted, for purposes of the disclosure, "gas" can include air, filtered or treated air, or other gases.

Throughout the disclosure, the terms "non-permeable", "gas-permeable", and "porous" can be used to describe properties (e.g., material properties, characteristics, parameters) of one or more features of a substrate.

Unless otherwise noted, for purposes of the disclosure, a "non-permeable" substrate (e.g., material of a non-permeable substrate) is considered to be impermeable to solid, liquid, and gas under normal conditions (e.g., no external influence including but not limited to pressure and force) and, therefore, does not permit the transfer of solid, liquid, or gas in to, through, or out of, the non-permeable substrate under normal conditions. In some embodiments, a non-permeable substrate can form a portion of the wall of the vessel. Additionally, the cell culture chamber of the vessel is considered to be sterile when a non-permeable substrate forms a portion of the wall of the vessel because bacteria, for example, cannot pass through the non-permeable substrate. However, when filling the plurality of microcavities of the substrate with material, gas can become trapped within the microcavity of a non-permeable substrate based on surface tension of the liquid, thereby, in some embodiments, preventing material from filling the microcavities and preventing growth of a spheroid.

Unless otherwise noted, for purposes of the disclosure, a "gas-permeable" substrate (e.g., material of a gas-permeable substrate) is considered to be impermeable to solid and liquid, and permeable to gas under normal conditions. Therefore, a gas-permeable substrate does not permit the transfer of solid and liquid in to, through, or out of, the gas-permeable substrate and does permit the transfer of gas in to, through, or out of, the gas-permeable substrate. In some embodiments, a gas-permeable substrate can form a portion of the wall of the vessel. Additionally, the cell culture chamber of the vessel is considered to be sterile when a gas-permeable substrate forms a portion of the wall of the vessel because bacteria, for example, cannot reasonably pass through the gas-permeable substrate. However, although the substrate is gas-permeable, gas can still become trapped in the microcavity during filling with material because gas-permeation rates through the gas-permeable substrate can be slower than the rate required to displace gas from the cavity under ordinary operating conditions and can therefore take an unacceptably long amount of time to permeate through the substrate. Thus, in some embodiments, slowly filling the microcavities allows the liquid front to enter each microcavity at an angle, thereby displacing gas as the liquid fills the microcavity. In some embodiments, after filling the cavity with liquid, gas can permeate (slowly) through the gas-permeable substrate.

Unless otherwise noted, for purposes of the disclosure, a "porous" substrate (e.g., material of a porous substrate) is considered to be impermeable to solid and permeable to liquid and gas under normal conditions. Therefore, a porous substrate does not permit the transfer of solid in to, through, or out of, the porous substrate and does permit the transfer of liquid and gas in to, through, or out of, the porous substrate. A porous substrate cannot form a portion of the vessel because bacteria can pass through a porous substrate, thus causing sterility issues in the cell culture chamber. Thus, when using a porous substrate, the substrate must be enclosed (entirely enclosed) in the sterile cell culture chamber of the vessel. During filling of the microcavities with material, however, gas can escape (e.g., pass) through the porous substrate. Thus, filling of the microcavities can be performed rapidly without concern for entrapping gas in the microcavities. In some embodiments, liquid can only pass through the porous substrate with added pressure or physical contact and disturbance of the substrate. Thus, in some embodiments, material including liquid can be contained in the microcavities of the substrate so long as the substrate is not exposed to added pressure or physical contact and disturbance. For example, in some embodiments, the porous substrate can be placed in the cell culture chamber to allow gas to pass through the substrate during filling as well as during culturing and to isolate the substrate from added pressure or physical contact and disturbance from external forces (e.g., outside the cell culture chamber).

A number of aspects of cell culture vessels and methods of culturing cells have been disclosed herein. A summary of some selected aspects is presented below.

In a first aspect, a cell culture vessel includes a base defining a base plane extending in a first direction and a second direction perpendicular to the first direction; a wall including an inner surface defining a cell culture chamber of the vessel; and a bottom surface of the cell culture chamber. The bottom surface of the cell culture chamber may be a separate substrate inserted into the cell culture chamber or may be a wall of the cell culture chamber. The substrate or bottom surface includes a plurality of microcavities, each microcavity of the plurality of microcavities includes a concave surface defining a well and an opening defining a path into the well. The substrate defines a substrate plane on which the opening of each microcavity of the plurality of microcavities is located, the substrate plane is oriented at a first angle relative to the first direction and at a second angle relative to the second direction, and an absolute value of at least one of the first angle and the second angle is greater than zero.

A second aspect is a cell culture vessel according to the first aspect, where an absolute value of the first angle is greater than zero, and an absolute value of the second angle is greater than zero.

A third aspect is a cell culture vessel according to aspect 1 or aspect 2, where an absolute value of a plane angle between the base plane and the substrate plane is from about 1° to about 30°.

A fourth aspect is a cell culture vessel according to aspect 3, where an absolute value of the plane angle is about 5°.

A fifth aspect is a cell culture vessel according to any one of aspects 1-4, including a first aperture extending through the wall in fluid communication with the cell culture chamber, and a second aperture extending through the wall in fluid communication with the cell culture chamber, where the second aperture is spaced from the first aperture along an outward direction extending away from the base plane and perpendicular to the base plane.

A sixth aspect is a cell culture vessel according to aspect 5, where the first aperture is spaced from the second aperture in the first direction.

A seventh aspect is a cell culture vessel according to aspect 5 or aspect 6, where the first aperture is spaced from the second aperture in the second direction.

In an eighth aspect, a method of culturing cells in the cell culture vessel of any one of aspects 1-7, includes depositing liquid in at least one microcavity of the plurality of microcavities, and culturing cells in the at least one microcavity after depositing the liquid in the at least one microcavity.

A ninth aspect is a method of culturing cells according to aspect 8, where the base plane is perpendicular relative to the direction of gravity while culturing the cells in the at least one microcavity.

A tenth aspect is a method of culturing cells according to aspect 8 or aspect 9, including flowing material in a flow direction parallel to the substrate plane while culturing the cells in the at least one microcavity.

In an eleventh aspect, a method of culturing cells in the cell culture vessel of any one of aspects 5-7 includes passing liquid through the first aperture from outside the vessel into the cell culture chamber, and flowing the liquid in the cell culture chamber along a flow direction parallel to the substrate plane.

A twelfth aspect is a method of culturing cells according to aspect 11, including passing gas through the second aperture from the cell culture chamber to outside the vessel while flowing the liquid in the cell culture chamber along the flow direction.

A thirteenth aspect is a method of culturing cells according to aspect 11 or aspect 12, including culturing cells in at least one microcavity of the plurality of microcavities while flowing the liquid in the cell culture chamber along the flow direction.

A fourteenth aspect is a method of culturing cells according to aspect 13, where the base plane is perpendicular relative to the direction of gravity while culturing the cells in the at least one microcavity.

In a fifteenth aspect, a method of culturing cells in the cell culture vessel of any one of aspects 5-7 includes flowing liquid in the cell culture chamber along a flow direction parallel to the substrate plane, and passing the liquid through the first aperture from the cell culture chamber to outside the vessel.

A sixteenth aspect is a method of culturing cells according to aspect 15, including passing gas through the second aperture from outside the vessel into the cell culture chamber while flowing the liquid in the cell culture chamber along the flow direction.

A seventeenth aspect is a method of culturing cells according to aspect 15 or aspect 16, including culturing cells in at least one microcavity of the plurality of microcavities while flowing the liquid in the cell culture chamber along the flow direction.

An eighteenth aspect is a method of culturing cells according to aspect 17, where the base plane is perpendicular relative to the direction of gravity while culturing the cells in the at least one microcavity.

In a nineteenth aspect, a method of culturing cells includes depositing liquid in at least one microcavity of a plurality of microcavities of a substrate positioned in a cell culture chamber of a vessel while a base plane of the vessel is oriented perpendicular relative to the direction of gravity, the base plane extending in a first direction and a second direction perpendicular to the first direction, where each microcavity of the plurality of microcavities includes a concave surface defining a well and an opening defining a path into the well, where the substrate defines a substrate plane on which the opening of each microcavity of the plurality of microcavities is located, where the substrate plane is oriented at a first angle relative to the first direction and at a second angle relative to the second direction, and an absolute value of at least one of the first angle and the second angle is greater than zero; culturing cells in the at least one microcavity after depositing the liquid in the at least one microcavity; and flowing material in a flow direction parallel to the substrate plane while culturing the cells in the at least one microcavity.

A twentieth aspect is a method of culturing cells according to aspect 19, where an absolute value of the first angle is greater than zero, and an absolute value of the second angle is greater than zero.

A twenty-first aspect is a method of culturing cells according to aspect 19 or aspect 20, where an absolute value of a plane angle between the base plane and the substrate plane is from about 1° to about 30°.

A twenty-second aspect is a method of culturing cells according to aspect 21, where an absolute value of the plane angle is about 5°.

A twenty-third aspect is a method of culturing cells according to any one of aspects 19-22, including passing liquid through a first aperture in a wall of the vessel from outside the vessel into the cell culture chamber and passing gas through a second aperture in the wall of the vessel from the cell culture chamber to outside the vessel.

A twenty-fourth aspect is a method of culturing cells according to aspect 23, including passing gas through the second aperture from outside the vessel into the cell culture chamber and passing liquid through the first aperture from the cell culture chamber to outside the vessel.

A twenty-fifth aspect is a method of culturing cells according to aspect 23 or aspect 24, where the first aperture is positioned at a lower elevation relative to the direction of gravity than the second aperture.

In a twenty-sixth aspect, a cell culture vessel includes a substrate including a plurality of microcavities; a wall, the substrate and an inner surface of the wall define a cell culture chamber of the vessel; an aperture extending through the wall in fluid communication with the cell culture chamber; a first portion of the inner surface positioned opposite the aperture along an axis of the vessel, the substrate spans a length of the cell culture chamber that extends along the axis of the vessel; a second portion of the inner surface extending from the aperture to the substrate; and a third portion of the inner surface extending from the first portion to the substrate.

A twenty-seventh aspect is a cell culture vessel according to the twenty-sixth aspect, where each microcavity of the plurality of microcavities includes a concave surface defining a well and an opening defining a path from the cell culture chamber into the well.

A twenty-eighth aspect is a cell culture vessel according to aspect 26 or aspect 27, where the first portion is substantially perpendicular to the axis of the vessel.

A twenty-ninth aspect is a cell culture vessel according to any one of aspects 26-28, where the first portion and the third portion define a non-planar boundary portion of the cell culture chamber.

A thirtieth aspect is a cell culture vessel according to any one of aspects 26-29, where the third portion includes a stepped profile.

A thirty-first aspect is a cell culture vessel according to any one of aspects 26-29, where the third portion includes an inclined profile.

A thirty-second aspect is a cell culture vessel according to any one of aspects 26-31, further including a baffle extending from the second portion of the inner surface, where the baffle includes a major surface obstructing a path defined between the aperture and the substrate.

In thirty-third aspect, a method of culturing cells in the cell culture vessel of any one of aspects 26-32 includes passing liquid through the aperture from outside the vessel into the cell culture chamber, thereby providing a predetermined amount of liquid in the cell culture chamber.

A thirty-fourth aspect is a method of culturing cells according to aspect 33, including containing the predetermined amount of liquid in a region of the cell culture chamber without liquid of the predetermined amount of liquid contacting one or more microcavities of the plurality of microcavities.

A thirty-fifth aspect is a method of culturing cells according to aspect 34, where liquid of the predetermined amount of liquid contacts the first portion and the third portion while containing the predetermined amount of liquid in the region of the cell culture chamber without liquid of the predetermined amount of liquid contacting one or more microcavities of the plurality of microcavities.

A thirty-sixth aspect is a method of culturing cells according to aspect 34 or aspect 35, where the axis of the vessel extends substantially in the direction of gravity while containing the predetermined amount of liquid in the region of the cell culture chamber without liquid of the predetermined amount of liquid contacting one or more microcavities of the plurality of microcavities.

A thirty-seventh aspect is a method of culturing cells according to any one of aspects 34-36, including moving the vessel after containing the predetermined amount of liquid in the region of the cell culture chamber without liquid of the predetermined amount of liquid contacting one or more microcavities of the plurality of microcavities to cause at least a portion of the predetermined amount of liquid to flow from the region over the substrate along the length of the cell culture chamber and deposit in at least one microcavity of the plurality of microcavities.

A thirty-eighth aspect is a method of culturing cells according to aspect 37, including culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the predetermined amount of liquid in the at least one microcavity.

A thirty-ninth aspect is a method of culturing cells according to aspect 38, where the axis of the vessel is substantially perpendicular relative to the direction of gravity while culturing cells in the at least one microcavity of the plurality of microcavities.

In a fortieth aspect, a method of culturing cells includes passing liquid through an aperture in a wall of a vessel from outside the vessel into a cell culture chamber of the vessel defined by an inner surface of the wall and a substrate including a plurality of microcavities, thereby providing a predetermined amount of liquid in a region of the cell culture chamber; and containing the predetermined amount of liquid in the region of the cell culture chamber without liquid of the predetermined amount of liquid contacting one or more microcavities of the plurality of microcavities.

A forty-first aspect is a method of culturing cells according to aspect 40, including moving the vessel to cause at least a portion of the predetermined amount of liquid to flow from the region over the substrate and deposit in at least one microcavity of the plurality of microcavities.

A forty-second aspect is a method of culturing cells according to aspect 41, including culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the predetermined amount of liquid in the at least one microcavity.

In a forty-third aspect, a cell culture vessel includes a substrate including a plurality of microcavities; a wall, the substrate and an inner surface of the wall define a cell culture chamber of the vessel; an aperture extending through the wall in fluid communication with the cell culture chamber; a first portion of the inner surface positioned opposite the aperture along an axis of the vessel, where the substrate spans a length of the cell culture chamber that extends along the axis of the vessel; a second portion of the inner surface extending from the aperture to the substrate; and a baffle extending from the second portion, where the baffle includes a major surface obstructing a path defined between the aperture and the substrate.

A forty-fourth aspect is a cell culture vessel according to aspect 43, where each microcavity of the plurality of microcavities includes a concave surface defining a well and an opening defining a path from the cell culture chamber into the well.

A forty-fifth aspect is a cell culture vessel according to aspect 43 or aspect 44, where the major surface of the baffle is substantially perpendicular to the axis of the vessel.

A forty-sixth aspect is a cell culture vessel according to aspect 43 or aspect 44, where the major surface of the baffle includes a convex profile.

A forty-seventh aspect is a cell culture vessel according to aspect 43 or aspect 44, where the major surface of the baffle includes a concave profile.

A forty-eighth aspect is a cell culture vessel according to any one of aspects 43-47, where at least a portion of a free end of the baffle is spaced a distance from the inner surface of the wall.

In a forty-ninth aspect, a method of culturing cells in the cell culture vessel of any one of aspects 43-48, includes adding material into the cell culture chamber by inserting a dispensing-port into the aperture, and then dispensing material from the dispensing-port into the cell culture chamber.

A fiftieth aspect is a method of culturing cells according to aspect 49, including culturing cells in at least one microcavity of the plurality of microcavities while dispensing material from the dispensing-port into the cell culture chamber.

A fifty-first aspect is a method of culturing cells according to aspect 49 or aspect 25, including removing material from the cell culture chamber by inserting a collecting-port into the aperture, and then collecting material from the cell culture chamber with the collecting-port.

A fifty-second aspect is a method of culturing cells according to aspect 51, including culturing cells in at least one microcavity of the plurality of microcavities while collecting material from the cell culture chamber with the collecting-port.

In a fifty-third aspect, a method of culturing cells includes inserting a dispensing-port into an aperture in a wall of a vessel; flowing material along a first flow path in a cell culture chamber of the vessel defined by an inner surface of the wall and a substrate including a plurality of microcavities by dispensing material from the dispensing-port, thereby adding material from outside the vessel into the cell culture chamber; and obstructing the flow of material along the first flow path.

What is claimed is:

1. A cell culture vessel comprising:

at least one cell culture chamber, having a bottom, a top and sidewalls;

a base defining a support plane extending in a first direction and a second direction perpendicular to the first direction;

wherein the bottom, the top and the sidewalls each have surfaces facing the inside of the cell culture chamber;

wherein at least the bottom surface of the cell culture chamber comprises an array of microcavities;

an inlet in a top of the vessel providing access to the cell culture chamber on one side of the vessel;

an outlet in a bottom of the vessel providing access to each cell culture chamber on the opposite side of the vessel;

wherein the bottom surface of the cell culture chamber defines a substrate plane which is at a first angle relative to the first direction of the substrate plane and a second angle relative to the second direction of the substrate plane; and,

wherein an absolute value of at least one of the first angle and the second angle is from about 1° to about 30°.

2. The cell culture vessel of claim **1**, wherein the absolute value of both the first angle and the second angle is from about 1° to about 30°.

3. The cell culture vessel of claim **1**, wherein an absolute value of the first plane angle is about 5°, wherein an absolute value of the second plane angle is about 5°, or the absolute value of both the first plane angle and the second plane angle is about 5°.

4. The cell culture vessel of claim **1**, wherein the inlet is in the top corner of the vessel.

5. The cell culture vessel of claim **4**, wherein the outlet is in a bottom corner of the vessel opposite the inlet top corner.

6. The cell culture vessel of claim **1**, further comprising at least two cell culture chambers stacked one above the other.

7. The cell culture vessel of claim **6**, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold, and each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold.

8. The cell culture vessel of claim **6**, wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

9. The cell culture vessel of claim **6**, wherein the bottom of each cell culture chamber comprises gas permeable, liquid impermeable material.

10. The cell culture vessel of claim **9**, wherein each cell culture chamber is separated from its next adjacent cell culture chamber by a tracheal space.

11. A method of culturing cells in the cell culture vessel of claim **1**, comprising:

introducing liquid containing cells and media in the cell culture chambers through the inlet;

allowing cells to settle into the microcavities by gravity; and

culturing cells.

12. The method of claim **11**, further comprising flowing liquid into the vessel through the inlet, the cell culture chamber(s), and out of the vessel through the outlet while culturing the cells.

13. The cell culture vessel of claim **1**, wherein the first direction of the support plane of the base is along the axis between the inlet and the outlet; and

wherein the second direction of the support plane of the base is perpendicular to the axis between the inlet and the outlet.

14. The cell culture vessel of claim **2**, wherein the inlet is in the top corner of the vessel.

15. The cell culture vessel of claim **3**, wherein the inlet is in the top corner of the vessel.

16. The cell culture vessel of claim **15**, wherein the outlet is in a bottom corner of the vessel opposite the inlet top corner.

17. The cell culture vessel of claim **1**, comprising at least two cell culture chambers stacked one above the other.

18. The cell culture vessel of claim **1**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold, and each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold; and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

19. The cell culture vessel of claim **1**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold; each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold; and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

20. The cell culture vessel of claim **3**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold; each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

21. The cell culture vessel of claim **14**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold, and each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

22. The cell culture vessel of claim **15**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold, and each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

23. The cell culture vessel of claim **16**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold, and each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

24. The cell culture vessel of claim **1**, wherein the inlet is in the top corner of the vessel.

25. The cell culture vessel of claim **24**, wherein the outlet is in a bottom corner of the vessel opposite the inlet top corner.

26. The cell culture vessel of claim **25**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold; each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold; and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

27. The cell culture vessel of claim **24**, comprising at least two cell culture chambers stacked one above the other, wherein the inlet is fluidly connected to each cell culture chamber via an inlet manifold; each cell culture chamber has an inlet opening between the cell culture chamber and the inlet manifold and wherein the outlet is fluidly connected to each cell culture chamber via an outlet manifold, and each cell culture chamber has an outlet opening between the cell culture chamber and the outlet manifold.

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